Synthesis and Physicochemical Characterization of the Process-Related Impurities of Eplerenone, an Antihypertensive Drug

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Abstract: Two unknown impurities were observed during the process development for multigram-scale synthesis of eplerenone (Inspra®). The new process-related impurities were identified and fully characterized as the corresponding (7β,11α,17α)-11-hydroxy- and (7α,11β,17α)-9,11-dichloroeplerenone derivatives 12a and 13. Seven other known but poorly described in the literature eplerenone impurities, including four impurities A, B, C and E listed in the European Pharmacopoeia 8.4 were also detected, identified and fully characterized. All these contaminants result from side reactions taking place on the steroid ring C of the starting 11α-hydroxy-7α-(methoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (12) and the key intermediate (7α,17α)-9(11)-enedister 7, including epimerization of the C-7 asymmetric center, oxidation, dehydration, chlorination and lactonization. The impurities were isolated and/or synthesized and fully characterized by infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR) and high-resolution mass spectrometry/electrospray ionization (HRMS/ESI). Their 1H- and 13C-NMR signals were fully assigned. The molecular structures of the eight impurities, including the new (7β,11α,17α)-11-hydroxy- and (7α,11β,17α)-9,11-dichloroeplerenone related substances 12a and 13, were solved and refined using single-crystal X-ray diffraction (SCXRD). The full identification and characterization of these impurities should be useful for the quality control and the validation of the analytical methods in the manufacture of eplerenone.

Keywords: selective aldosterone blocker; hypertension; steroids; eplerenone; impurities; spectroscopic methods; crystal structure

1. Introduction

Eplerenone (2, Figure 1) is a cardiovascular drug indicated for the treatment of essential hypertension and congestive heart failure that, in contrast to its predecessor spironolactone 1 (Figure 1) demonstrates a high degree of selectivity for the aldosterone receptor and a low-binding affinity for progesterone and androgen receptors [1–8]. As a result of the presence of a 9,11-epoxide group in the eplerenone structure, its selectivity for the aldosterone receptor is enhanced and the drug minimizes the risk of adverse hormonal effects and provides important clinical benefits not previously available with spironolactone 1. Treatment with eplerenone is associated with reductions in blood pressure and improved survival (15% reduction in total mortality) for patients with heart failure who are in stable condition after a myocardial infarction. The product was originally developed by scientists at...
Ciba-Geigy AG (Basel, Switzerland) and launched in the US in 2003 by Pharmacia (Sandwich, Kent, UK) under the trade name Inspra®.

Eplerenone contains sensitive 9,11α-epoxide, 17α-γ-lactone and 7α-carbomethoxy moieties that, depending on the conditions, may hydrolyze or epimerize to afford degradation or epimerization products [9–13]. The SCXRD structure of eplerenone confirms the relative cis configuration of the 9α,11α-epoxide ring and the 7α-carbomethoxy substituent [14–17]. Manufacture of eplerenone is always accompanied by side reactions leading to the unwanted impurities that vary with the different starting materials and reaction conditions used. Most of the patents and literature information dealing with the preparation of eplerenone are based on the use of canrenone derivatives. As stereogenic centers in eplerenone precursors give rise to various process-related impurities, including diastereomers and regioisomers of starting materials, intermediates, by-products and the final drug substance, the manufacture of eplerenone with the required stereochemistry and pharmaceutical grade purity is a significant challenge. In designing a synthesis of eplerenone from canrenone derivatives, the principal challenges are the stereoselective introduction of the carbomethoxy substituent at the C-7 position of the steroid skeleton and the regioselective dehydration of the 11α-hydroxy group [1,9,16–21].

In 1984, Grob et al. [17,18] from Ciba-Geigy AG accomplished the first synthesis of eplerenone by employing Nagata hydrocyanation of Δ<sup>9(11)</sup>-canrenone (3) as the key step (Scheme 1), but with moderate stereoselectivity in the 7α-cyano derivative 4 formation (7α/7β ≈ 4:1) necessitating tedious column chromatographic separations. The original EP 122232 B1 patent does not disclose any information with respect to the purity of the material obtained, its purification to pharmaceutical-grade or the removal of impurities.

**Scheme 1.** The original eplerenone synthesis from Ciba-Geigy AG (Basel, Switzerland) [17,18]. Reagents and Conditions: (1) Et<sub>2</sub>AlCN, THF; (2) Dibal-H, benzene; (3) CrO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, acetone; (4) CH<sub>2</sub>N<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>; (5) m-CPBA, CH<sub>2</sub>Cl<sub>2</sub> or H<sub>2</sub>O<sub>2</sub>, Cl<sub>3</sub>CCN, K<sub>2</sub>HPO<sub>4</sub>, CH<sub>2</sub>Cl<sub>2</sub>.
In alternative eplerenone synthesis by Ng et al. [9], the 7α-carbomethoxy group was introduced stereoselectively via 4,6-bishydrocyanation of 11α-hydroxycanrenone (9, Scheme 2), but regioselective dehydration of the intermediate (7α,11α,17α)-11-hydroxyester 12 was problematic giving the regioisomeric (7α,17α)-11(12)-epoxyester 7b apart from the main (7α,17α)-9(11)-enester 7 product (Table 1). The 7α/7β diastereomeric purity of (7α,11α,17α)-12 obtained by the cleavage of diketone 11 with sodium methoxide in refluxing methanol was not reported; however, the literature sources on syntheses of related 7α-carboxalkoxy stereoidal spirrolactones provide information on their 7β-carboxalkoxy diastereoisomers resulting from basic epimerization of the steroid C-7α asymmetric center under the same conditions as used for diketone 11 cleavage [22].

When the authors repeated the preparation taught in WO 9825948 A2 it was observed that the purity of the resulting eplerenone was low and attempts to purify the resulting product to a level meeting the requisite specifications for use as a pharmaceutical active were unsuccessful. Although the epimeric 11-hydroxyester (7β,11α,17α)-12a was not reported by Ng et al. [9], or any other literature sources, it contaminated the main (7α,17α)-9(11)-enester 7 product. In particular, the regioisomeric (7α,11α,12α,17α)-11,12-epoxyester 2b (Imp. E), the new (7α,11β,17α)-9,11-dichloro derivative 13 and the 7α,9,21,17-dicarbolactone 14 (Imp. A) were difficult to remove from the final product. All these contaminants resulted from side reactions taking place on the steroid ring C of the (7α,11α,17α)-11-hydroxy derivative (12) and the key intermediate (7α,17α)-9(11)-enester 7, including epimerization of the C-7α asymmetric center, oxidation, dehydration, chlorination and lactonization.

Recently, we have developed an improved, scalable, cost-effective and environment-friendly technology for the industrial-scale synthesis of eplerenone (2) from commercially available 11α-hydroxy-7α-(methoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (12), based on the last two steps of the general route described by Ng et al. (Scheme 2) [9]. During the process development, two new and seven known process-related impurities of eplerenone were observed, and/or synthesized and fully characterized, including four impurities A, B, C and E listed in the European Pharmacopoeia 8.4 [23]. The new impurities were identified as 11α-hydroxy-7β-(methoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (12a) and 9,11β-dichloro-7α-(methoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (13). To the best of our knowledge, the 11-hydroxyester 12a and the 9,11-dichloro derivative 13 are new compounds that have never been identified before.

The syntheses of the starting (7α,11α,17α)-11-hydroxyester 12 and the key intermediate (7α,17α)-9(11)-enester 7 are broadly described in the literature sources [9,16–21,24]; however, their

**Scheme 2.** Synthesis of EP from G. D. Searle & Co. (Chicago, IL, USA) [9]. **Reagents and Conditions:**
(1) *Aspergillus ochraceus*; (2) LiCl, DMF, Et3N, Me2CO, (CH3)2C(OH)CN; (3) HCl, MeOH, H2O; (4) MeONa, MeOH; (5) SO2Cl2, ImH, THF; (6) H2O2, K2HPO4, C5HCONH2.

![Diagram of Scheme 2](image-url)
comprehensive structure elucidation and confirmation is still missing and SCXRD studies are reported here for the first time. As starting materials and intermediates in active pharmaceutical ingredient (API) synthesis often afford numerous impurities affecting the quality of the final drug product, their comprehensive structural elucidation and confirmation is essential for impurities identification and characterization. A complete physicochemical characterization, not only for an API, but also starting materials and key synthetic intermediates, became a requirement of both the U.S. Food and Drug Administration (FDA) and the European Medicine Agency (EMA). The five other impurities listed in Table 1, i.e., the isomeric (7β,17α)-9(11)-enester 7a and (7α,17α)-11(12)-enester 7b, the isomeric (7β,11α,17α)-9,11-epoxyester 2a (Imp. E) and (7α,11α,12α,17α)-11,12-epoxyester 2b (Imp. B) and the 7α,9,21,17-dicarbolactone 14 (Imp. A), were mentioned elsewhere in the literature, mainly as part of HPLC studies; however, their syntheses, methods of removal from the final product and comprehensive structural elucidation and confirmation were not disclosed [9,12]. The determination of a drug substance impurity profile, including starting materials, by-products, intermediates and potential degradation products, is critical for the safety assessment of API and manufacturing process thereof. In any API, it is necessary to study the impurity profile, and control it during the preparation of the pharmaceutical. As indicated in the ICH guidelines, any impurity, formed at a level of ≥0.10% with respect to the API, should be identified, synthesized and characterized thoroughly [25,26]. Only two eplerenone impurities, i.e., 7α,9,21,17-dicarbolactone 14 (Imp. A) and 11,12-epoxy ester 2b (Imp. B), are accepted at a level greater than 0.10%, i.e., maximum 0.3%, in accordance with pharmacopoeial acceptance criteria [22].

Table 1. Structures of the process-related impurities of eplerenone (2).

| Chemical Structure | Name |
|--------------------|------|
| ![Image](image1.png) | 11α-hydroxy-7α-(methoxycarbonyl)-3-oxo-17α-pregna-4,11(12)-diene-21,17-carbolactone |
| ![Image](image2.png) | 11α-hydroxy-7β-(methoxycarbonyl)-3-oxo-17α-pregna-4,11(12)-diene-21,17-carbolactone |
| ![Image](image3.png) | 7α-(methoxycarbonyl)-3-oxo-17α-pregna-4,9(11)-diene-21,17-carbolactone |
| ![Image](image4.png) | 7β-(methoxycarbonyl)-3-oxo-17α-pregna-4,9(11)-diene-21,17-carbolactone |
| ![Image](image5.png) | 7α-(methoxycarbonyl)-3-oxo-17α-pregna-4,11(12)-diene-21,17-carbolactone |
| ![Image](image6.png) | 7α-(methoxycarbonyl)-3-oxo-17α-pregna-4,11(12)-diene-21,17-carbolactone |
Table 1. Cont.

| Chemical Structure | Name                                                      |
|--------------------|-----------------------------------------------------------|
| ![Chemical Structure](image1.png) | 9,11β-dichloro-7α-(methoxycarbonyl)-3-oxo-17α-pregn-4-en-21,17-carbolactone |
| ![Chemical Structure](image2.png) | 3-oxo-17α-pregn-4-en-7α,9,21,17-dicarbolactone             |
| ![Chemical Structure](image3.png) | 9,11α-epoxy-7β-(methoxycarbonyl)-3-oxo-17α-pregn-4-en-21,17-carbolactone |
| ![Chemical Structure](image4.png) | 11α,12α-epoxy-7α-(methoxycarbonyl)-3-oxo-17α-pregn-4-en-21,17-carbolactone |
| ![Chemical Structure](image5.png) | 2b (Imp. B) $^1$                                         |

$^1$ Impurities listed in the European Pharmacopoeia 8.4 [23].

The present paper deals with synthesis, physicochemical characterization and comprehensive structural elucidation and confirmation of the process-related eplerenone impurities found in samples of the key intermediate (7α,17α)-9(11)-enester 7 and eplerenone bulk drug substance manufactured according to the modified and optimized procedure described in Searle Co. patent [9] (Scheme 2, 12 as starting material). Apart from the starting material 12, the key intermediate enester 7 and the final product 2, seven process-related eplerenone impurities 2a-b, 7a-b, 12a, 13 and 14 were fully characterized by IR, NMR and MS. The molecular structures of the epimeric 11-hydroxyesters (7α,11α,17α)-12 and (7β,11α,17α)-12a, the isomeric (7α,17α)-9(11)-enester 7, (7β,17α)-9(11)-enester 7a and (7α,17α)-11(12)-enester 7b, the isomeric (7β,11α,17α)-9,11-epoxyester 2a and (7α,11α,12α,17α)-11,12-epoxyester 2b, and the new (7α,11β,17α)-9,11-dichloro derivative 13, were solved and refined using SCXRD. The full identification and characterization of these compounds should be useful for the quality control and the validation of the analytical methods in the manufacture of eplerenone.

2. Results and Discussion

Dehydration of 11-hydroxy steroids is commonly used for the introduction of the 9,11-double bond into the steroid skeleton. In a preferred technological embodiment, the (7α,17α)-9(11)-enester 7 is synthesized directly by in situ replacement of the 11α-hydroxy group of the ester (7α,11α,17α)-12 with halogen followed by thermal 9,11-dehydrohalogenation. The nucleophilic substitution of the 11α-hydroxy group is effected by reaction with sulfuryl halide at about −70 °C, after which a hydrogen halide scavenger is added. In manufacturing technology of eplerenone developed by the authors, the 11α-hydroxyester 12 and imidazole were dissolved simultaneously in anhydrous tetrahydrofuran and cooled to −10 °C. Sulfuryl chloride was added slowly and the reaction mixture was allowed to warm to room temperature, and then stirred for a time sufficient to complete the elimination reaction, typically about 1 h. The key intermediate 7 was isolated in a crude form by dichloromethane extraction followed by evaporation of the solvent and recrystallized twice, from ethanol and a mixture...
of dichloromethane/diethyl ether respectively, to give the pure (7α,17α)-9(11)-enester 7 (71% yield) as white crystals.

The dehydration step produced the three main side products, i.e., the regioisomeric (7β,17α)-11(12)-enester 7b, the new (7α,11β,17α)-9,11-dichloro derivative 13 and the 7α,9:21,17-dicarbolactone 14 (Imp. A), which were isolated chromatographically from the recrystallization mother liquors of 7 by elution with varying mixtures of ethyl acetate/dichloromethane. Further gradient elution with acetonitrile/toluene afforded the new 11-hydroxyester (7β,11α,17α)-12a, which was isolated in only a small yield, apart from the unreacted (7α,11α,17α)-12 isomer used as the starting material.

The commercial 11-hydroxyester (7α,11α,17α)-12 was synthesized according to the procedure described by Ng et al. [9] and the chemical purity declared by the manufacturer was 98%. The novel 11-hydroxyester (7β,11α,17α)-12a was not previously detected by chromatographic or spectroscopic methods in its commercially available (7α,11α,17α)-12 isomer. However, the literature data on syntheses of related 7α-carboalkoxy steroidal spiranlactones [22] allowed us to assume that the new 11-hydroxyester (7β,11α,17α)-12a originates from the fourth step of the Scheme 2 synthesis in which sodium methoxide reacts with diketone 11 to afford the (7α,11α,17α)-12 isomer as the main product of the cleavage, which is contaminated with the (7β,11α,17α)-12a epimer resulting from the competitive basic epimerization of 12. The 11-hydroxyester (7β,11α,17α)-12a was synthesized independently by basic epimerization of the (7α,11α,17α)-12 isomer. As expected, sodium methoxide in methanol solution converted the C-7α ester 12 into an epimeric mixture of the C-7α 12 and C-7β 12a esters. The pure C-7β ester 12a was isolated chromatographically from the crude mixture of epimers 12 and 12a by 10–50% acetonitrile/toluene gradient elution to give (7β,11α,17α)-12a (49% yield) as a white solid. A sample of 12a was also isolated by column chromatography from recrystallization mother liquors of the commercial 11-hydroxyester (7α,11α,17α)-12. Even a small amount of the newly detected (7β,11α,17α)-11-hydroxyester 12a is hardly separable from its (7α,11α,17α)-12 isomer by large scale chromatography or recrystallization, therefore, the commercial 11-hydroxyester (7α,11α,17α)-12 was used further without purification. As the two 11-hydroxyesters of the C-7α and C-7β series, respectively 12 and 12a, are crucial for impurities formation during multi-gram scale synthesis of eplerenone, they were subjected to comprehensive structural elucidation and confirmation.

The [M + Na]+ values, m/z 439.2110 and 439.2080, obtained for both 11-hydroxyesters 12 and 12a correspond to C29H32O5Na. The NMR data for epimeric hydroxyesters 12 and 12a are given in Table 2. The 1H-/13C-NMR chemical shifts assignment was made after careful and precise 2D NMR spectra analysis and the data obtained for the known isomer 12 are in full compliance with those presented by Preisig et al. [24]. The 2D NOESY experiments allowed discrimination between epimeric structures 12 and 12a and fully confirmed the stereochemistry at the C-7 atom in both isomers. Blue arrows in Figure 2 show the most important NOE effects involving H7 proton, simultaneously clearly indicating the 7α or 7β positioned carboxethoxy group. The strong H7-H8 NOE effect is observed for the compound 12 with β positioned H7 proton, whereas a situated H7 proton in 12a is involved in two significant H9-H7 and H7-H14 NOE effects. Additionally, the strong NOE effect between H9 and H14 protons for both 12 and 12a epimers is observed. Comparison of the NMR data for epimeric structures 12 and 12a revealed that some of the 1H/13C nuclei shieldings within the steroid rings B, C and D are related to the α or β configuration of the C-7 atom. The 1H shielding increase of 0.5 ppm for H7, 0.71 ppm for H9 and 0.28 ppm for H14 nuclei is observed when passing from the compound 12 with 7α positioned carboxethoxy group to its 7β epimer 12a. Additionally, the change of configuration at the C7 is accompanied by H8 (0.35 ppm) shielding decrease. Simultaneously, both diastereotopic H15 protons of the epimer 12a with 7β positioned carboxethoxy group became equal having the same proton chemical shift (1.49 ppm). In the case of 13C-NMR data the opposite effect is observed, the transition from 12 to 12a leads to the shielding decrease of 6.6 ppm for C7, 4.8 ppm for C9 and 3.4 ppm for C14 nuclei. Surprisingly, the change of configuration from 7α in 12 to 7β in 12a is related with no change of the shielding of the C8 nucleus and decreasing of shielding by 2.7 ppm for C23 carbon of the carboxethoxy substituent.
was the main product of the 11-hydroxyester 12 dehydration with sulfuryl chloride in the presence of

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**Figure 2.** Crucial 1H- and 13C-NMR chemical shifts and NOE effects for 11-hydroxyesters: (a) (7α,11α,17α)-12; (b) (7β,11α,17α)-12a.

**Table 2.** The 1H- and 13C-NMR spectral data in CDCl3 for 11-hydroxyesters (7α,11α,17α)-12 and (7β,11α,17α)-12a.

|   | 1H-NMR (1) | 13C-NMR  |
|---|------------|----------|
|   | 12         | 12a      |
|   | Carbon     | C-1      | 37.3   | 37.1   |
| H-1a | 2.02 m     | 2.07 m   | C-2    | 34.1   | 34.2   |
| H-1b | 2.80 m     | 2.71 m   | C-3    | 199.5  | 199.9  |
| H-2a | 2.34 m     | 2.33 m   | C-4    | 126.1  | 125.0  |
| H-2b | 2.46 m     | 2.43 m   | C-5    | 167.7  | 167.2  |
| H-4 | 5.69 s     | 5.75 s   | C-6    | 36.3   | 37.3   |
| H-6a | 2.52 m     | 2.37 m   | C-7    | 42.4   | 49.0   |
| H-6b | 2.64 ddd (1.9, 5.8, 14.6) | 2.62 m | C-8    | 37.0   | 37.0   |
| H-8 | 1.79 m     | 2.14 m   | C-9    | 52.6   | 57.4   |
| H-9 | 1.93 m     | 1.22 t (10.4) | C-10  | 39.4   | 39.6   |
| H-11 | 4.04 m     | 4.10 m   | C-11   | 69.2   | 68.5   |
| H-12a | 1.42 m     | 1.42 m   | C-12   | 43.4   | 43.5   |
| H-12b | 1.85 m     | 1.83 dd (4.6, 11.6) | C-13  | 46.2   | 46.6   |
| H-14 | 1.65 m     | 1.37 m   | C-14   | 45.3   | 48.7   |
| H-15a | 1.41 m     | 1.49 m   | C-15   | 22.5   | 22.5   |
| H-15b | 1.73 m     | 1.49 m   | C-16   | 35.4   | 35.6   |
| H-16a | 1.88 m     | 1.80 ddd (6.9, 8.8, 14.3) | C-17  | 95.0   | 94.5   |
| H-16b | 2.26 ddd (3.6, 12.1, 14.5) | 2.21 ddd (4.4, 11.4, 14.3) | C-18  | 15.4   | 16.1   |
| H-18 | 1.01 s     | 1.03 s   | C-19   | 18.4   | 18.4   |
| H-19 | 1.36 s     | 1.40 s   | C-20   | 31.2   | 31.4   |
| H-20a | 1.95 m     | 1.93 ddd (7.5, 9.4, 13.0) | C-21  | 29.1   | 29.2   |
| H-20b | 2.39 m     | 2.37 m   | C-22   | 176.5  | 176.5  |
| H-21a | 2.50 m     | 2.49 ddd (11.6, 13.1, 17.7) | C-23  | 172.7  | 175.4  |
| H-21b | 2.57 m     | 2.56 ddd (6.2, 9.3, 17.7) | C-24  | 51.6   | 52.1   |
| H-24 | 3.65 s     | 3.69 s   |        |        |        |

1 Values in parentheses give δ, multiplicity and J in Hz.

The NMR structure assignments of the isomeric 11-hydroxyesters (7α,11α,17α)-12 and (7β,11α,17α)-12a were confirmed by X-ray analysis (Figure 3 and Table 3). The SCXRD structures confirmed the relative cis- and trans-configurations of the 11α-hydroxy and 7α-carbomethoxy substituents in 12 and 12a, respectively. The carbomethoxy group is situated at the C-7α position in 12 and the C-7β position in 12a, whereas the hydroxy group adopts the C-11α position in both 12 and 12a isomers. The dehydration of 11α-hydroxy steroids leads predominantly to the formation of the double bond between C-9 and C-11 carbons of the steroid skeleton. As expected, the (7α,17α)-9(11)-enester 7 was the main product of the 11-hydroxyester 12 dehydration with sulfuryl chloride in the presence of...
imidazole; however, a considerable amount of the regioisomeric \((7\alpha,17\alpha)-11(12)\)-enester \(7b\), as a result of competitive 11,12-dehydration of 12, was also isolated from the recrystallization mother liquors of 7.

Figure 3. Molecular structure of the 11-hydroxysterest: (a) \((7\alpha,11\alpha,17\alpha)-12\) and (b) \((7\beta,11\alpha,17\alpha)-12a\).

Table 3. Summary of crystallographic data and structure refinement for the 11-hydroxysterest \((7\alpha,11\alpha,17\alpha)-12\) and \((7\beta,11\alpha,17\alpha)-12a\).

| Identification Code | 12       | 12a      |
|---------------------|----------|----------|
| Chemical formula    | \(C_{24}H_{32}O_6\) | \(C_{24}H_{32}O_6\) |
| Molecular weight    | 416.50 g/mol | 416.50 g/mol |
| Temperature         | 100(2) K  | 100(2) K  |
| Wavelength          | 0.71073 Å | 0.71073 Å |
| Crystal system, space group | Orthorhombic, P2\(_{1}\)2\(_{1}\)2\(_{1}\) | Orthorhombic, P2\(_{1}\)2\(_{1}\)2\(_{1}\) |
| Unit cell dimensions |          |          |
| \(a\) = 6.7560(2) Å | \(a\) = 12.2862(3) Å |
| \(b\) = 16.1436(5) Å | \(b\) = 12.4532(3) Å |
| \(c\) = 19.0947(6) Å | \(c\) = 13.3060(4) Å |
| \(\alpha = 90^\circ\) | \(\alpha = 90^\circ\) |
| \(\beta = 90^\circ\) | \(\beta = 90^\circ\) |
| \(\gamma = 90^\circ\) | \(\gamma = 90^\circ\) |
| Volume              | 2082.59(11) Å\(^3\) | 2035.85(9) Å\(^3\) |
| \(Z\), Calculated density | 4,132 mg/m\(^3\) | 4,139 mg/m\(^3\) |
| Absorption coefficient | 0.094 | 0.096 |
| \(F(000)\)          | 896      | 896      |
| Crystal size        | 0.30 × 0.20 × 0.20 mm | 0.30 × 0.20 × 0.10 mm |
| Theta range for data collection | 2.13°–25.68° | 2.26°–25.67° |
| Limiting indices    |          |          |
| \(-8 \leq h \leq 8\) | \(-14 \leq h \leq 14\) |
| \(-19 \leq k \leq 19\) | \(-15 \leq k \leq 15\) |
| \(-23 \leq l \leq 23\) | \(-16 \leq l \leq 16\) |
| Reflections collected/unique | 21,220/3973 | 11,318/3856 |
| Absorption correction | semi-empirical from equivalents | semi-empirical from equivalents |
| Max. and min. transmission | 0.9814 and 0.99723 | 0.9904 and 0.9716 |
| Refinement method    | full-matrix least-squares on \(F^2\) | full-matrix least-squares on \(F^2\) |
| Data/restraints/parameters | 3973/0/275 | 3856/1/275 |
| Goodness-of-fit on \(F^2\) | 1.156 | 1.041 |
| Final \(R1/wR2\) indices \([I > 2\sigma(I)]\) | 0.0360/0.0921 | 0.0311/0.0721 |
| \(R1/wR2\) indices (all data) | 0.0481/0.1132 | 0.0361/0.0756 |
| Largest diff. peak and hole | 0.191 and \(-0.341\) e/Å\(^3\) | 0.172 and \(-0.212\) e/Å\(^3\) |
Additionally, the 9,11-dehydration of the residual 11-hydroxyester (7β,11α,17α)-12a contaminating the starting 12 isomer afforded the epimeric (7β,17α)-9(11)-ene ester 7a, which was also isolated chromatographically from recrystallization mother liquors of 7. It was synthesized independently by epimerization of the (7α,17α)-9(11)-ene ester 7 with sodium methoxide in methanol, or dehydration of the 11-hydroxyester (7β,11α,17α)-12a with sulfuryl chloride and imidazole, and then purified by column chromatography with 5–20% ethyl acetate/dichloromethane gradient elution to give the pure (7β,17α)-9(11)-ene ester 7a, with 37% or 85% yield respectively, as a white solid.

The [M + Na]+ values, m/z 421.1986, 421.2006, and 421.1988, obtained for the free isomeric enesters 7, 7a and 7b correspond to C₂₄H₃₀O₅Na. The full NMR data confirming the structure of the known enester 7 were reported by Grob et al. [17]; however several ¹H and ¹³C signals were assigned reversibly. The proper ¹H/¹³C chemical shift assignment for isomeric enesters 7, 7a and 7b was based on the careful analysis of 2D NMR experiments and is given in Table 4. Similarly to the epimeric hydroxyesters 12 and 12a, the NOE effects involving H7 proton allowed distinction between 7α (7 and 7b) and 7β (7a) diastereoisomers (Figure 4). The strong H7-H8 NOE effect was noted for isomers 7 and 7b with β positioned H7 proton, whereas α situated H7 in 7a is involved in H7-H14 NOE effect disturbed by H7-H15 interaction. Additionally, the significant H9-H14 NOE effect for 7b regiosomer was observed, while H8 proton of the epimers 7 and 7b is involved in H8-H6 interaction. Similarly to the epimeric hydroxyesters 12 and 12a, some of the ¹H/¹³C nuclei shieldings within the steroid rings B, C and D are related to the α or β configuration of the C-7 atom. The ¹H shielding increase of 0.7 ppm for H7 and 0.22 ppm for H8 nuclei was observed when passing from the compound 7 with 7α positioned carbomethoxy group to its 7β epimer 7a. Similarly to 12a, both diastereotopic H15 protons of the epimer 7a with β positioned carbomethoxy group became equal having the same proton chemical shift (1.54 ppm). In the case of ¹³C-NMR the opposite effect is observed, the transition from 7 to 7a leads to the shielding decrease of 5.9 ppm for C7 and 2.4 ppm for C14 nuclei. The change of configuration from 7α in 7 to 7β in 7a is related with no change of the shielding of the C8 nucleus and decreasing of shielding by 2.3 ppm for C23 carbon of carbomethoxy substituent. The dehydration of the epimeric 11-hydroxyesters 12/12a to the corresponding 9(11)-enesters 7/7a caused the strong ¹H shielding decrease of 89.7 ppm/84.7 ppm for C9 and 49.8 ppm/51.9 ppm for C11 carbons, respectively. The transition from 12/12a to 7/7a also resulted in medium shielding increase of 10.5/10.8 ppm for C12 and shielding decrease of 8.8/7.3 ppm for C19 and 3.4/3.4 ppm for C8, whereas for C10 (1.1/1.3 ppm), C13 (1.7/1.9 ppm) and C14 (2.2/3.2 ppm) a weak increasing effect was observed. The competitive dehydration of 12 to the regiosomer 11,12-enester 7b resulted in the strong ¹H shielding decrease of 57.3 ppm for C11 and 90.4 ppm for C12 nuclei. Similarly to the hydroxyesters 12 and 12a, the change of configuration from 7α in 7 to 7β in 7a is related with no change of the shielding of the C8 nucleus and decreasing of shielding by 2.3 ppm for C23 carbon of the carbomethoxy substituent. Additionally, the shielding increase of 4.5 ppm for C8 carbon was observed when passing from epimeric 9(11)-enesters 7 and 7a to regiosomer 11-enester 7b.

![Figure 4. Crucial ¹H- and ¹³C-NMR chemical shifts and NOE effects for esters: (a) (7α,17α)-9(11)-ene 7; (b) (7β,17α)-9(11)-ene 7a; (c) (7α,17α)-11-ene 7b.](image-url)
The carbomethoxy group is situated at the C-7 position in SCXRD structures confirmed the presence of the 9,11-double bond in (7α,17α)-9(11)-ene 7α and (7α,17β)-11-ene 7b.

| Proton | 7  | 7a | 7b | Carbon | 7  | 7a | 7b |
|--------|----|----|----|--------|----|----|----|
| H-1a   | 2.18 m | 2.12 m | 1.87 m | C-1 | 33.7 | 33.9 | 34.9 |
| H-1b   | 2.24 m | 2.12 m | 2.15 m | C-2 | 34.2 | 34.2 | 33.7 |
| H-2a   | 2.53 m | 2.50 m | 2.45 m | C-3 | 198.6 | 198.6 | 198.7 |
| H-2b   | 2.53 m | 2.50 m | 2.45 m | C-4 | 125.7 | 124.6 | 126.8 |
| H-4    | 5.71 d (2.0) | 5.75 d (2.0) | 5.81 s | C-5 | 166.6 | 165.5 | 166.3 |
| H-6a   | 2.55 m | 2.43 dd (3.8, 14.6) | 2.67 m | C-6 | 35.7 | 36.2 | 35.6 |
| H-6b   | 2.82 ddd (2.0, 5.3, 15.0) | 2.85 ddd (2.0, 13.2, 14.6) | 2.67 m | C-7 | 43.8 | 40.7 | 42.1 |
| H-7    | 2.98 m | 2.28 m | 2.89 m | C-8 | 40.4 | 40.4 | 35.9 |
| H-8    | 2.48 m | 2.70 m | 2.24 m | C-9 | 142.3 | 141.2 | 49.2 |
| H-9    | -    | -    | 2.44 m | C-10 | 40.5 | 40.9 | 38.8 |
| H-11   | 5.65 m | 5.68 m | 5.59 dd (2.1, 10.2) | C-11 | 119.0 | 120.4 | 126.5 |
| H-12a  | 1.87 m | 1.88 m | 2.49 m | C-12 | 32.9 | 32.7 | 133.8 |
| H-12b  | 2.20 m | 2.17 m | - | C-13 | 44.5 | 44.7 | 49.0 |
| H-14   | 1.49 m | 1.50 m | 1.95 m | C-14 | 43.1 | 45.5 | 44.1 |
| H-15a  | 1.49 m | 1.54 m | 1.57 m | C-15 | 23.3 | 23.8 | 21.8 |
| H-15b  | 1.89 m | 1.54 m | 1.87 m | C-16 | 35.4 | 35.4 | 35.7 |
| H-16a  | 1.87 m | 1.81 m | 1.95 m | C-17 | 95.1 | 94.5 | 93.9 |
| H-16b  | 2.31 m | 2.26 m | 2.20 m | C-18 | 14.1 | 14.3 | 18.6 |
| H-18   | 0.95 s | 0.96 s | 1.05 s | C-19 | 27.2 | 25.7 | 17.3 |
| H-19   | 1.39 s | 1.42 s | 1.16 s | C-20 | 31.4 | 31.2 | 32.3 |
| H-20a  | 1.92 m | 1.93 m | 2.00 m | C-21 | 29.2 | 29.1 | 28.7 |
| H-20b  | 2.29 m | 2.30 m | 2.21 m | C-22 | 176.5 | 174.6 | 176.7 |
| H-21a  | 2.50 m | 2.51 m | 2.61 m | C-23 | 172.6 | 174.9 | 173.0 |
| H-21b  | 2.59 dddd (6.0, 9.4, 17.8) | 2.60 m | 2.53 m | C-24 | 51.4 | 52.1 | 51.6 |
| H-24   | 3.59 s | 3.72 s | 3.66 s |

1 Values in parentheses give δ, multiplicity and J in Hz.

The NMR structure assignments of the isomeric (7α,17α)-9(11)-ene ester 7α and (7α,17α)-11-ene ester 7b were confirmed by X-ray analysis (Figure 5 and Tables 5 and 6). The SCXRD structures confirmed the presence of the 9,11-double bond in 7α and the 11,12-double bond in 7b. The carbomethoxy group is situated at the C-7α position in 7α and 7b and at the C-7β position in 7b.

![Figure 5. Molecular structure of the isomeric 9(11)-enesters: (a) (7α,17α)-7 and (b) (7β,17α)-7a.](image)

| Identification Code | 7b | 13 |
|---------------------|----|----|
| Chemical formula    | C2H5O2 | C2H7Cl2O5 |
| Molecular weight    | 398.48 g/mol | 470.39 g/mol |
| Temperature         | 100(2) K | 100(2) K |
| Wavelength          | 0.71073 Å | 0.71073 Å |
| Crystal system, space group | Orthorhombic, P212121 | Orthorhombic, P212121 |
| Unit cell dimensions | a = 6.2322(5) Å, b = 15.8874(13) Å, c = 20.5238(15) Å | a = 9.2383(4) Å, b = 14.2496(6) Å, c = 16.3898(7) Å |
|                     | α = 90°, β = 90°, γ = 90° | α = 90°, β = 90°, γ = 90° |

Table 5. Summary of crystallographic data and structure refinement for (7α,17α)-11(12)-ene ester 7b and (7α,11β,17α)-9,11-dichloro derivative 13.
### Table 5. Cont.

| Identification Code | 7b | 13 |
|---------------------|----|----|
| Volume              | 2032.1(3) Å³ | 2157.99(16) Å³ |
| Z, Calculated density | 4,1302 mg/m³ | 4,1448 mg/m³ |
| Absorption coefficient | 0.090 | 0.336 |
| F(000)              | 856 | 996 |

| Crystal size | 0.30 × 0.15 × 0.15 mm | 0.30 × 0.10 × 0.10 mm |
| Limiting indices | -2 ≤ h ≤ 7 | -11 ≤ h ≤ 11 |
| | -19 ≤ k ≤ 19 | -17 ≤ k ≤ 17 |
| | -25 ≤ l ≤ 25 | -19 ≤ l ≤ 19 |
| Reflections collected/unique | 35,771/4149 | 21,917/4092 |
| Absorption correction | semi-empirical from equivalents | semi-empirical from equivalents |
| Max. and min. transmission | 0.9866 and 0.9739 | 0.9671 and 0.9058 |
| Data/restraints/parameters | 4149/0/265 | 4092/0/283 |
| Goodness-of-fit on F² | 1.017 | 1.024 |
| Final R₁/wR² indices [I > 2σ(I)] | 0.0319/0.0883 | 0.0368/0.0698 |
| Final R₁/wR² indices (all data) | 0.0337/0.0903 | 0.0515/0.0749 |
| Largest diff. peak and hole | 0.295 and -0.135 e/Å³ | 0.231 and -0.244 e/Å³ |

### Table 6. Summary of crystallographic data and structure refinement for the isomeric 9(11)-enesters (7α,17α)-7 and (7β,17α)-7a.

| Identification Code | 7 | 7a |
|---------------------|----|----|
| Chemical formula    | C₂₄H₃₀O₅ | C₂₄H₃₀O₅ |
| Molecular weight    | 398.48 g/mol | 398.48 g/mol |
| Temperature         | 100(2) K | 100(2) K |
| Wavelength          | 0.71073 Å | 0.71073 Å |
| Crystal system, space group | Orthorhombic, P₂₁2₁2₁ | Orthorhombic, P₂₁2₁2₁ |
| Unit cell dimensions | a = 6.296(2) Å | a = 12.099(3) Å |
|                     | b = 15.468(3) Å | b = 12.310(3) Å |
|                     | c = 21.088(3) Å | c = 13.497(3) Å |
|                     | α = 90° | α = 90° |
|                     | β = 90° | β = 90° |
|                     | γ = 90° | γ = 90° |
| Volume              | 2053.7(8) Å³ | 2010.2(8) Å³ |
| Z, Calculated density | 4,1289 mg/m³ | 4,1317 mg/m³ |
| Absorption coefficient | 0.089 | 0.091 |
| F(000)              | 856 | 856 |
| Crystal size        | 0.41 × 0.32 × 0.29 mm | 0.60 × 0.39 × 0.31 |
| Theta range for data collection | 3.183°–28.719° | 3.310°–36.896° |
| Limiting indices    | -8 ≤ h ≤ 8 | -15 ≤ h ≤ 20 |
|                     | -19 ≤ k ≤ 19 | -19 ≤ k ≤ 20 |
|                     | -27 ≤ l ≤ 28 | -22 ≤ l ≤ 22 |
| Reflections collected/unique | 14,340/4925 | 36,105/9538 |
| Absorption correction | None | None |
| Max. and min. transmission | 0.975 and 0.964 | 0.972 and 0.947 |
| Refinement method   | full-matrix least-squares on F² | full-matrix least-squares on F² |
| Data/restraints/parameters | 4925/0/262 | 9538/0/262 |
| Goodness-of-fit on F² | 0.929 | 0.975 |
| Final R₁/wR² indices [I > 2σ(I)] | 0.0475/0.1011 | 0.0484/0.1205 |
| R₁/wR² indices (all data) | 0.0673/0.1071 | 0.0622/0.1259 |
| Largest diff. peak and hole | 0.389/–0.220 e/Å³ | 0.453/–0.303 e/Å³ |
Sulfuryl chloride used for dehydration of 11α-hydroxy steroids is also known as a chlorinating agent and, under some conditions, applied for chlorination of steroid double bonds [27,28]. Indeed, the novel (7α,11β,17α)-9,11-dichloro derivative 13, formed as a result of competitive chlorine addition to the newly formed 9,11-double bond of the key intermediate (7α,17α)-9(11)-enester 7, was isolated chromatographically from the recrystallization mother liquors of 7. It was also synthesized by chlorination of the pure enester 7 with sulfuryl chloride in the presence of pyridine in chlorobenzene, and then purified by column chromatography with 5–30% EtOAc/CH2Cl2 gradient elution to give the pure (7α,11β,17α)-9,11-dichloro derivative 13 (41% yield) as a white solid. The novel 9,11-dichloro impurity 13 was formed by the nucleophilic attack of a chloride anion on the intermediate chloronium cation, a structural analogue of the eplerenone epoxide ring. No other isomers of 13 were obtained.

The [M + Na]+ value, m/z 491.1386 obtained for the 9,11-dichloroderivative 13 corresponds to C24H30O5NaCl2. The stereochemistry at the C9 and C11 carbons of the dichloro derivative 13 was established on the basis of 1D/2D NOESY experiments, which indicated the relative trans configuration of the C-9α and C-11β positioned chlorine atoms. The addition of chlorine to the 9,11-double bond of enester 7 caused significant changes in the 1H/13C nuclei shieldings within the steroid rings A, B, C and D of the dichloro derivative 13 (Table 7).

Table 7. The 1H and 13C-NMR spectral data in CDCl3 for (7α,11β,17α)-9,11-dichloro derivative 13 and 7α,9:21,17-dicarbolactone 14.

| Proton  | 1H-NMR | 13C-NMR |
|---------|--------|---------|
|        |        |         |         |        |         |
| H-1a    | 2.27 m | 1.88 m  | C-1     | 29.6   | 29.6    |
| H-1b    | 2.77 m | 2.27 m  | C-2     | 33.8   | 33.2    |
| H-2a    | 2.50 m | 2.49 m  | C-3     | 197.8  | 197.1   |
| H-2b    | 2.50 m | 2.49 m  | C-4     | 125.7  | 129.4   |
| H-4     | 5.89 d (1.8) | 5.86 d (2.0) | C-5 | 163.7 | 161.5 |
| H-6a    | 2.60 m | 2.65 dd (4.0, 15.4) | C-6 | 32.4  | 34.5    |
| H-6b    | 2.93 m | 2.80 d (15.4) | C-7 | 40.9  | 45.4    |
| H-7     | 2.83 m | 2.68 m  | C-8     | 41.1   | 45.1    |
| H-8     | 2.90 m | 2.44 d (11.2) | C-9 | 86.6  | 90.0    |
| H-9     |        |        | C-10    | 47.1   | 43.7    |
| H-11a   | 4.68 dd (2.0, 4.8) | 1.84 m  | C-11    | 59.5  | 22.4    |
| H-11b   | 2.05 ddd (2.0, 4.7, 15.2) | -       | C-12    | 37.0  | 26.9    |
| H-12a   | 1.98 m | 1.56 m  | C-13    | 45.0   | 44.0    |
| H-12b   | 2.54 m | 1.61 m  | C-14    | 41.3   | 42.7    |
| H-14    | 2.46 m | 1.33 m  | C-15    | 23.6   | 23.4    |
| H-15a   | 1.57 m | 1.49 m  | C-16    | 35.3   | 35.2    |
| H-15b   | 2.32 m | 1.84 m  | C-17    | 95.9   | 94.8    |
| H-16a   | 1.97 m | 1.92 m  | C-18    | 17.2   | 13.4    |
| H-16b   | 2.33 m | 2.31 m  | C-19    | 26.1   | 19.1    |
| H-18    | 1.30 s | 0.99 s  | C-20    | 31.5   | 31.0    |
| H-19    | 1.86 s | 1.40 s  | C-21    | 29.1   | 29.0    |
| H-20a   | 2.06 m | 1.91 m  | C-22    | 176.3  | 176.2   |
| H-20b   | 2.39 m | 2.35 ddd (3.6, 5.6, 13.0) | C-23 | 172.3  | 176.5   |
| H-21a   | 2.48 m | 2.60 ddd (6.7, 9.2, 17.7) | C-24 | 52.2  | -       |
| H-21b   | 2.60 m | 2.51 m  | -       |        |         |

1 Values in parentheses give δ, multiplicity and J in Hz.

The strong shielding increase of 55.7 ppm for C9 and 59.5 ppm for C11 nuclei was observed when passing from 7 to 13. Minor shielding increase of 4.1 ppm for C1, 2.9 ppm for C5, 3.3 ppm for C6, 2.9 ppm for C7 and 1.8 ppm for C14 and shielding decrease of 6.6 ppm for C10 nuclei were also noted. The introduction of chlorine into C9 and C11 positions of 7 also resulted in shielding increase of 0.97 ppm for H11 and shielding decrease of 0.18 ppm for H4, 0.42 ppm for H8 and 0.97 for H14.
nuclei in dichloro derivative 13. Noteworthy, the diasterotopic effect observed for H15 protons of 7 (0.4 ppm) increased to 0.75 ppm for the same protons in 13. Similarly to the other compounds with 7α-carbomethoxy group, the β positioned H7 proton in dichloro derivative 13 is involved in strong H7-H8 NOE effect (Figure 6). The NMR structure assignment of the novel (7α,11β,17α)-9,11-dichloro derivative 13 was confirmed by X-ray analysis (Figure 7 and Table 5). The SCXRD structure confirmed the presence of the two chlorine atoms at the C-9α and C-11β positions of the steroid ring C in the relative trans configuration and the C-7α positioned carbomethoxy substituent.

**Figure 6.** Crucial 1H- and 13C-NMR chemical shifts and NOE effects for: (a) (7α,11β,17α)-9,11-dichloro derivative 13; (b) 7α,9,21,17-dicarbolactone 14.

**Figure 7.** Molecular structure of (a) (7α,17α)-11-enester 7b and (b) (7α,11β,17α)-9,11-dichloro derivative 13.

The acidic conditions applied for the dehydration of the 11α-hydroxy group in 12 resulted in competitive lactonization between the hydroxyl and carbomethoxy groups and lead to 7α,9,21,17-dicarbolactone 14 (Imp. A), which tends to be formed even in the presence of traces of free water. The dicarbolactone 14 was isolated chromatographically from the recrystallization mother liquors of 7. It was also synthesized independently from the mesylate of the starting 11α-hydroxyester 12 by reaction with acetic acid in the presence of sodium acetate, and then purified by column chromatography with 5–30% ethyl acetate/dichloromethane gradient elution to afford the pure 7α,9,21,17-dicarbolactone 14 (83% yield) as a white solid.

The [M + Na]+ value, m/z 407.1847, obtained for the dicarbolactone 14 corresponds to C23H28O5Na. The 1H-/13C-NMR chemical shifts assignment for dilactone 14 is presented in Table 7. The detailed analysis of 2D NMR spectra, especially 1H-13C HMBC and NOESY experiments, unambiguously
confirmed the structure consisting of the two γ-lactone rings. The second γ-lactone moiety results from internal lactonization between 9α-hydroxy and 7α-carbomethoxy groups of intermediate ester, formed by water addition to the 9,11-double bond of enester 7 during the dehydration step of 12. This significant change in the steroid structure entails numerous changes in the 1H/13C nuclei shielding. The shielding increase of 46.8 ppm for C11 and 16.5 ppm for C12 and decrease of 37.4 ppm for C9 nuclei were observed when passing from hydroxyester 12 to the dicarbolactone 14. Minor shielding increase of 7.7 ppm for C1 and 6.2 ppm for C5 was also noted, whereas for C8 shielding decrease of 8.1 ppm was observed. Similarly to the other compounds of the series with 7α positioned carbomethoxy group, the H7 proton of the dicarbolactone 14 is involved in two strong H7-H8 and H6-H7 NOE effects (Figure 6).

The purification of the key intermediate (7α,17α)-9(11)-enester 7 by large-scale recrystallization only partially removes the impurities formed during the dehydration of the starting 11α-hydroxyester 12. Thus, in a preferred technological embodiment, the key intermediate enester 7 was isolated in a crude form by dichloromethane extraction followed by evaporation of the solvent, and used directly in the following step of the process, which was the epoxidation of the 9,11-double bond to produce the eplerenone. Thus, the crude enester 7 was dissolved in dichloromethane and epoxidized with hydrogen peroxide in the presence of disodium hydrogen phosphate and trichloroacetamide. The crude product was recrystallized from ethanol and 2-butanone to give the pharmaceutical grade eplerenone. Apart from the (7α,11β,17α)-9,11-dichloro derivative 13 and the 7α,9,21,17-dicarbolactone 14 (Imp. A), which were present in the crude enester 7, the epoxidation step produced the two further impurities, i.e., the isomeric (7β,11α,17α)-9,11-epoxyester 2a and (7α,11α,12α,17α)-11,12-epoxyester 2b, which were isolated chromatographically from the recrystallization mother liquors of 2. The trichloroacetamide is known to preferentially epoxidize the more highly substituted double bond, e.g., the 9,11-olefin. In the case of the crude 9,11-enester 7 contaminated with the 11,12-enester 7b, the 9,11-epoxide should be formed with the minimal competitive epoxidation of the 7b isomer yielding the 11,12-epoxide as the by-product. As expected, the chromatography of the recrystallization mother liquors of 2 with 1–10% acetone/dichloromethane gradient elution gave the (7α,11α,12α,17α)-11,12-epoxyester 2b, which was undoubtedly formed via epoxidation of the (7α,17α)-9(11)-enester 7b. It was also synthesized independently by epoxidation of the enester 7b with hydrogen peroxide in the presence of disodium hydrogen phosphate and trichloroacetamide, and purified by column chromatography to afford the pure 11,12-epoxyester 2b (60% yield) as a white solid. Further gradient elution of the recrystallization mother liquors of 2 with 1–10% acetone/dichloromethane gave the isomeric (7β,11α,17α)-9,11-epoxyester 2a, which was formed by epoxidation of the (7β,17α)-9(11)-enester 7a contaminating the crude intermediate enester 7. It was also synthesized independently by epoxidation of the enester 7a with hydrogen peroxide in the presence of disodium hydrogen phosphate and trichloroacetamide, or basic epimerization of eplerenone (2), and then purified by column chromatography to give the pure (7β,11α,17α)-9,11-epoxyester 2a, with 69% or 49% yield respectively, as a white solid. The recrystallization of the crude eplerenone (2) from ethanol removes the new 9,11-dichloro derivative 13, the isomeric (7β,11α,17α)-9,11-epoxyester 2a and the residuals of the unreacted isomeric (7β,17α)-9(11)-enester 7a and (7α,17α)-9(11)-enester 7b. The recrystallization from 2-butanone removes the 7α,9,21,17-dicarbolactone 14 (Imp. A) and affords the pharmaceutical grade eplerenone (2).

The NMR data of eplerenone were presented in literature sources many times; however the full and correct 1H/13C chemical shifts assignment confirming its structure has never been done. Although the complete assignment of 1H-/13C-NMR signals was presented by Grob et al. [17], some of the signals were assigned reversibly. The careful analysis of 2D NMR experiments, including HSQC, HMBC and NOE/SY measurements, allowed to assign signals to the corresponding protons and carbons of the isomeric epoxy esters 2, 2a and 2b unambiguously, and thus confirm the structure of eplerenone and its isomers (Table 8). Blue arrows in Figure 8 show the most important NOE effects involving H7 proton, simultaneously indicating the 7α or 7β positioned carbomethoxy group. The strong H7-H8 NOE effect is observed for the compounds 2 and 2b with β positioned H7 proton, whereas α situated H7 proton in 2a is involved in the strong interaction with H14. The epoxidation of the 9,11-double bond caused several shielding
effects observed for $^1$H/$^{13}$C nuclei within the steroid rings A, B and C. The strong shielding increase of 77.1/75 ppm for C9, 67.6/66.8 ppm for C11 carbons and 2.5/2.4 ppm for H11 protons was observed when passing from enesters 7/7a to epoxides 2/2a, respectively. Minor shielding increase of 7/6.5 ppm for C1, 5.9/6.1 ppm for C14, 2.6/3.4 for C7, 1.8/1.6 ppm for C8, 2/2 ppm for C12 and 0.7/0.4 for C13 carbons was also noted. The transition from enesters 7/7a to epoxides 2/2a caused shielding increase of 0.76/0.66 ppm for one of the H1 protons and 0.36/0.31 ppm for one of the H12 protons, whereas for H-14 protons shielding decrease of 0.4/0.14 ppm was noted. Similarly to the epimeric hydroxyesters 12/12a and enesters 7/7a, some of the $^1$H/$^{13}$C nuclei shielding within the steroid rings B, C and D are related to the $\alpha$ or $\beta$ configuration of the C7 atom. The $^1$H shielding increase of 0.22 ppm for H7 and 0.25 ppm for H14 protons was observed when passing from the compound 2 with $\alpha$ positioned carbomethoxy group to its $\beta$ epimer 2a. Additionally, the change of configuration at the C7 is accompanied by weak shielding decrease of 0.1 ppm for H8. Simultaneously, both diasterotopic H15 protons of the epimer 2a with $\beta$ positioned carbomethoxy group became equal having the same proton chemical shift (1.51 ppm). In the case of $^{13}$C-NMR data the opposite effect is observed, the transition from 2 to 2a leads to the shielding decrease of 5.1 ppm for C7, 1.9 ppm for C9 and 2.2 ppm for C14 nuclei. The change of configuration from $\alpha$ in 2 to $\beta$ in 2a is also related with shielding decrease of 2.1 ppm for C23 carbon of the carbomethoxy substituent. The competitive epoxidation of the 11,12-enester 7b to the 11,12-epoxyester 2b resulted in the strong shielding increase of 76.1 ppm for C11 and 77.1 ppm for C12 nuclei, whereas for C9 (3.3 ppm), C18 (4.4 ppm) and C14 (6.7 ppm) medium effects were observed. The strong shielding increase of 2.57 and 2.84 ppm was noted for H11 and H12 protons, respectively. Minor changes in increasing of shielding for H7 (0.1 ppm), H8 (0.24 ppm) and H9 (0.4 ppm) protons were also observed. Similarly to the enesters 7, 7a, and 7b, the shielding increase of 3.6 ppm and 3.8 ppm for C8 carbon was noted when passing from epimeric 9,11-epoxides 2 and 2a to regioisomeric 11,12-epoxyester 2b.

Table 8. $^1$H- and $^{13}$C-NMR spectral data for (7$^\alpha$,11$^\alpha$,17$^\alpha$)-9,11-epoxyester 2, (7$^\beta$,11$^\alpha$,17$^\alpha$)-9,11-epoxyester 2a and (7$^\alpha$,11$^\alpha$,12$^\alpha$,17$^\alpha$)-11,12-epoxyester 2b.

| Proton | 2 | 2a | 2b | Carbon | 2 | 2a | 2b |
|--------|---------------|---------------|---------------|-------|---------------|---------------|---------------|
| H-1a   | 1.42 m        | 1.46 ddd (2.8, 5.2, 13.2) | 2.02 m        | C-1   | 26.7     | 27.4     | 34.7     |
| H-1b   | 2.19 m        | 2.07 m        | 2.30 ddd (2.6, 5.0, 13.4) | C-2   | 33.0    | 33.2    | 33.4    |
| H-2a   | 2.43 m        | 2.39 m        | 2.47 ddd (0.8, 2.6, 5.0, 17.5) | C-3   | 197.9   | 197.9   | 198.2   |
| H-2b   | 2.47 m        | 2.44 m        | 2.52 ddd (5.0, 14.3, 17.5) | C-4   | 127.0   | 126.8   | 126.6   |
| H-3    | 3.89 s        | 5.83 d (1.8)  | 5.81 s        | C-5   | 165.0   | 163.1   | 165.3   |
| H-4a   | 2.72 m        | 2.90 m        | 2.64 m        | C-6   | 34.8     | 36.2     | 35.5     |
| H-4b   | 2.72 m        | 2.89 ddd (2.2, 12.7, 15.3) | 2.64 m | C-7   | 41.2    | 46.3     | 41.7     |
| H-7    | 2.89 m        | 2.67 ddd (3.9, 11.2, 12.6) | 2.79 m | C-8   | 38.6    | 38.8     | 35.0     |
| H-8    | 2.49 ddd (4.7, 11.2) | 2.59 m | 2.00 m | C-9   | 65.2    | 67.1     | 45.9     |
| H-9    | -            | -            | 2.04 m        | C-10  | 39.7    | 40.0     | 38.2     |
| H-11   | 3.12 d (5.4)  | 3.28 d (5.4)  | 3.02 d (4.0)  | C-11  | 51.4    | 53.6     | 50.4     |
| H-12a  | 1.70 d (5.4, 14.6) | 1.72 ddd (5.4, 14.6) | 3.05 d (4.0)  | C-12  | 30.9    | 30.7     | 56.7     |
| H-12b  | 1.84 d (5.4, 14.6) | 1.86 d (14.6) | -            | C-13  | 43.8    | 44.3     | 47.2     |
| H-15a  | 1.89 m        | 1.64 m        | 1.92 m        | C-14  | 37.2    | 39.4     | 37.4     |
| H-15b  | 1.51 m        | 1.51 m        | 1.51 m        | C-15  | 22.0    | 22.4     | 21.2     |
| H-15c  | 2.03 m        | 1.51 m        | 1.90 m        | C-16  | 34.9    | 35.1     | 34.5     |
| H-16a  | 1.88 m        | 1.76 m        | 1.90 m        | C-17  | 94.5    | 93.9     | 94.0     |
| H-16b  | 2.51 m        | 2.15 m        | 2.18 m        | C-18  | 16.1    | 16.5     | 14.2     |
| H-18   | 1.02 s        | 1.02 s        | 1.09 s        | C-19  | 22.2    | 21.0     | 17.9     |
| H-19   | 1.50 s        | 1.54 s        | 1.30 s        | C-20  | 30.8    | 30.9     | 31.9     |
| H-20a  | 1.94 ddd (7.0, 9.6, 13.2) | 1.89 m  | 2.00 m | C-21  | 28.8    | 29.0     | 28.8     |
| H-20b  | 2.31 ddd (6.5, 9.5, 13.2) | 2.28 ddd (6.7, 9.5, 13.2) | 2.73 ddd (7.5, 8.9, 12.9) | C-22  | 176.1   | 176.1   | 176.7   |
| H-21a  | 2.50 ddd (6.9, 9.4, 17.9) | 2.50 m  | 2.60 m | C-23  | 172.5   | 174.6    | 172.5    |
| H-21b  | 2.60 ddd (6.7, 9.6, 17.9) | 2.60 m  | 2.60 m | C-24  | 51.5    | 52.2     | 51.7     |
| H-24   | 3.65 s        | 3.71 s        | 3.66 s        |       |         |          |          |

1 Values in parentheses give $\delta$, multiplicity and $J$ in Hz.
The [M + Na]+ values, m/z 437.1941 and 437.1942, obtained for the two isomeric epoxyesters 2 and 2b correspond to C24H30O6Na. The [M + H]+ value, m/z 415.2113, obtained for the third isomeric epoxyester 2a corresponds to C24H31O6. The NMR structure assignments of the isomeric (7β,11α,17α)-9,11-epoxyester 2a and (7α,11α,12α,17α)-11,12-epoxyester 2b were confirmed by X-ray analysis (Figure 9 and Table 9). The SCXRD structures confirmed the presence of the 9α,11α-epoxide ring in 2a and 11α,12α-epoxide ring in 2b. The carbomethoxy group is situated at the C-7α position in 2b and at the C-7β position in 2a.

![Diagram](image_url)

**Figure 8.** Crucial 1H- and 13C-NMR chemical shifts and NOE effects for: (a) (7α,11α,17α)-9,11-epoxyester 2; (b) (7β,11α,17α)-9,11-epoxyester 2a and (c) (7α,11α,12α,17α)-11,12-epoxyester 2b.

![Diagram](image_url)

**Figure 9.** Molecular structure of the isomeric epoxides: (a) (7β,11α,17α)-9,11-epoxy-2a and (b) (7α,11α,12α,17α)-11,12-epoxy-2b.

| Identification Code | 2a | 2b |
|---------------------|----|----|
| Chemical formula    | C24H30O6 | C24H31O6 |
| Molecular weight    | 414.48 g/mol | 414.48 g/mol |
| Temperature         | 100(2) K | 100(2) K |
| Wavelength          | 0.71073 Å | 0.71073 Å |
| Crystal system, space group | Monoclinic, P2₁ | Orthorhombic, P2₁2₁2₁ |
| Unit cell dimensions | a = 6.0294(5) Å | a = 6.377(2) Å |
|                      | b = 10.5733(8) Å | b = 15.477(3) Å |
|                      | c = 16.3548(12) Å | c = 20.886(4) Å |
|                      | α = 90° | α = 90° |
|                      | β = 99.60(2)° | β = 90° |
|                      | γ = 90° | γ = 90° |

**Table 9.** Summary of crystallographic data and structure refinement for the isomeric (7β,11α,17α)-9,11-epoxyester 2a and (7α,11α,12α,17α)-11,12-epoxyester 2b.
### Table 9. Cont.

| Identification Code | 2a                        | 2b                        |
|---------------------|---------------------------|---------------------------|
| Volume              | 1028.0(14) Å³             | 2061.4(9) Å³              |
| Z, Calculated density | 2, 1.339 mg/m³           | 4, 1.336 mg/m³           |
| Absorption coefficient | 0.095                     | 0.095                     |
| F(000)              | 444                       | 888                       |
| Crystal size        | 0.30 × 0.20 × 0.02 mm     | 0.60 × 0.27 × 0.12 mm     |
| Theta range for data collection | 2.30°–27.48°             | 3.21°–25.23°              |
| Limiting indices    | −7 ≤ h ≤ 7               | −7 ≤ h ≤ 7               |
|                     | −13 ≤ k ≤ 13             | −18 ≤ k ≤ 18             |
|                     | −21 ≤ l ≤ 21             | −25 ≤ l ≤ 24             |
| Reflections collected/unique | 36785/4712            | 9774/3635                 |
| Absorption correction | semi-empirical from equivalents | None                   |
| Max. and min. transmission | 0.9981 and 0.9720         | 0.989 and 0.945           |
| Refinement method   | full-matrix least-squares on F² | full-matrix least-squares on all F² |
| Data/restraints/parameters | 4712/1/274             | 3635/0/271                |
| Goodness-of-fit on F² | 1.093                     | 1.057                     |
| Final R1/wR2 indices [I > 2sigma(I)] | 0.0391/0.1014         | 0.0371/0.0942             |
| R1/wR2 indices (all data) | 0.0408/0.1024         | 0.0427/0.0964             |
| Largest diff. peak and hole | 0.261 and −0.179 e/Å³     | 0.220 and −0.192 e/Å³     |

### 3. Experimental Section

#### 3.1. General Information

11α-Hydroxy-7α-(methoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (12, 98%) was manufactured by Hangzhou Pharma Chemicals Ltd. (Hangzhou, China) according to synthetic procedure described by Ng et al. [9] (Scheme 2). Sulfuryl chloride (97%), imidazole (99%), 2,2,2-trichloroacetamide (99%), disodium hydrogen phosphate (≥99%), hydrogen peroxide (35% in H₂O) were purchased from Sigma-Aldrich (Munich, Germany) and CHEMPUR (Piekary Ślaskie, Poland) chemical companies. Deionized water was prepared using a MilliQ plus purification system (Millipore, Bradford, PA, USA). Potassium bromide (FT-IR grade) and deuterated chloroform were purchased from Merck KGaA (Darmstadt, Germany). The course of all reactions and the purity of products were checked by thin-layer chromatography (TLC). Analytical TLC was performed on silica gel DC-Alufolien Kieselgel 60 F₂₅₄ (Merck KGaA), with mixtures of toluene, ethyl acetate, acetone, dichloromethane and acetonitrile, in various ratios as developing systems. Compounds were detected by spraying the plates with 1% Ce(Ⅲ)SO₄/2%H₂O[P(Mo₃O₁₀)₄] in 10% H₂SO₄ followed by heating to 120 °C. Column chromatography was carried out on silica gel (Kieselgel 60, 40–63 μm, 230–400 mesh, Merck) with mixtures of ethyl acetate, toluene, acetone, acetonitrile and dichloromethane in varying ratios as eluents.

#### 3.2. Optical Rotation

Optical rotations were measured with a Perkin Elmer 341 automatic polarimeter (Perkin Elmer, Norwalk, CT, USA) in CH₂Cl₂ solutions as the solvents with percent concentrations.

#### 3.3. Melting Point

Melting points were determined with a MEL-TEMP II capillary melting point apparatus (Laboratory Devices, Holliston, MA, USA).
3.4. FT-IR Spectroscopy

FT-IR spectra were taken for KBr pellets on a Nicolet Impact 410 FT-IR spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

3.5. NMR Spectroscopy

The NMR spectra of all the compounds were measured in CDCl$_3$ solutions with a Varian VNMRS-600 (600 MHz for $^1$H-NMR and 150 MHz for $^{13}$C-NMR; Varian Inc., Palo Alto, CA, USA) at temperature 298 K using TMS as internal standard. All $^1$H-/$^{13}$C-NMR resonance signals were assigned using results of 2D experiments [g-COSY ($^1$H-$^1$H), g-HSQC ($^1$H-$^{13}$C) and g-HMBC ($^1$H-$^{13}$C)] in gradient versions. The $^1$H-NMR chemical shifts were determined as centres of the correlation spots in the $^1$H domain of the 2D $^1$H-$^{13}$C HSQC experiments. Relative configuration and stereochemistry at the C-7 and other atoms was established on the basis of 1D and 2D NOESY spectra. Concentration of all solutions used for measurements was about 20–30 mg of compounds in 0.6 mL of solvent.

3.6. Mass Spectrometry

HRMS spectra were recorded on an AMD 604 Inectra GmbH (AMD Inectra GmbH, Harpstedt, Germany) and a Mariner PE Biosystem ESI-TOF (PerSeptive Biosystems/Applied Biosystems, Waltham, MA, USA) spectrometers.

3.7. X-ray Analysis

The X-ray diffraction data for 2b, 7, 7b, 12, 12a and 13 were collected using a KM4CCD-axis diffractometer (Kuma Diffraction, Wroclaw, Poland) with graphite-monochromated MoK radiation and equipped with an nitrogen gas-flow apparatus (Oxford Cryosystems, Oxford, UK). The data were corrected for Lorentz and polarization effects. The multi-scan absorption correction was applied for 2b, 12a and 13. Data reduction and analysis were carried out with the Oxford Diffraction Ltd. (Wroclaw, Poland) suit of programs [29,30]. The structures were solved by direct methods approach using the SHELXS97 [31] program and refined with the SHELXL97 [32]. The X-ray diffraction data for 2a and 7b were collected using the Kappa APEX II Ultra (Bruker, Billerica, MA, USA) controlled by APEX II software [33], equipped with MoKα rotating anode X-ray source ($\lambda = 0.71073$ Å, 50.0 kV, 22.0 mA) monochromatized by multi-layer optics and APEX-II CCD detector. The experiments were carried out at 100 K using the Oxford Cryostream cooling device. Indexing, integration and initial scaling were performed with SAINT [34] and SADABS [35] software. The structures were solved by direct methods approach using the SHELXS97 [31] program and refined with the SHELXL97 [32]. Multi-scan absorption correction has been applied in the scaling procedure. Crystal data and refinement details for 2b, 7, 7a-b, 12, 12a and 13 are listed in Tables 3, 5, 6 and 9. The deposition numbers CCDC 1502629 (2a), CCDC 1565292 (2b), CCDC 1565294 (7), CCDC 1565293 (7a), CCDC 1502628 (7b), CCDC 1502627 (12), CCDC 1502631 (12a) and CCDC 1502630 (13) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44-1223-336-033; E-mail: deposit@ccdc.cam.ac.uk).

3.8. Syntheses

3.8.1. 11α-Hydroxy-7β-(methoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (12a, Scheme 3)

11-Hydroxyester (7α,11α,17α)-12 (5.0 g, 12.0 mmol) was added to a suspension of MeONa (1.30 g, 24.0 mmol) in anhydrous MeOH (25 mL) and then refluxed for 24 h. The mixture was cooled to room temperature and 3 M aqueous HCl solution (150 mL) was added. The product was extracted with CH$_2$Cl$_2$ (3 × 25 mL). The combined organic phases were dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure to give a mixture of epimers 12 and 12a as a yellowish
white foam. The crude product was purified by column chromatography over silica gel with 10–50% MeCN/PhCH₃ gradient elution to give (7β,11α,17α)-12a (2.45 g, 49% yield) as a white solid, a mixture of 12 and 12a (0.56 g) and the (7α,11α,17α)-12 (1.27 g).

Scheme 3. Synthesis of the 11-hydroxyester (7β,11α,17α)-12a.

(7β,11α,17α)-12a: Rf = 0.40 for 60% MeCN/PhCH₃. M.p. 250–252 °C. [α]D²₀ = +21.49 (c 1.0, CHCl₃). FT-IR (pellets, KBr) ν (cm⁻¹): 3511, 2978, 2940, 2872, 2832, 1775, 1736, 1652, 1608, 1482, 1447, 1417, 1361, 1300, 1275, 1170, 1107, 1047, 1003, 914, 789, 742, 672, 596, 509, 470. HRMS (ESI): calcd. for C₂₄H₃₂O₆Na [M + Na]+ 439.2091, found 439.2080.

(7α,11α,17α)-12: Rf = 0.33 for 60% MeCN/PhCH₃. M.p. 228–229 °C (lit. 229–230 °C [24]). [α]D²₀ = +40.68 (c 1.0, CHCl₃) (lit. [α]D²₀ = +18.9 (c 0.0027, CHCl₃) [24]). FT-IR (pellets, KBr) ν (cm⁻¹): 3422, 3035, 2985, 2954, 2933, 2897, 2877, 1759, 1719, 1662, 1617, 1469, 1443, 1376, 1269, 1234, 1211, 1181, 1144, 1059, 1017, 999, 926, 866, 852, 787, 677, 606, 533, 514, 466. HRMS (ESI): calcd. for C₂₄H₃₂O₆Na [M + Na]+ 439.2091, found 439.2080. ¹H- and ¹³C-NMR spectra, see Table 2.

3.8.2. Purification of the Commercial (7α,11α,17α)-11-hydroxyester 12 and Isolation of the (7β,11α,17α)-11-hydroxyester 12a Impurity

The commercial 11-hydroxyester (7α,11α,17α)-12 (32.5 g) was purified by recrystallization from a mixture of AcOEt/MeCN/CH₂Cl₂ (8:4:1) to afford the pure 12 (13.98 g, 43% yield) as a white crystals. The recrystallization mother liquors of 12, containing a mixture of epimers 12 and 12a, were subjected to column chromatography over silica gel with 10–50% MeCN/PhCH₃ gradient elution to give (7β,11α,17α)-12a (0.91 g) as a white solid, a mixture of 12 and 12a (14.4 g) and (7α,11α,17α)-12 (2.67 g). The characterization data of 12 and 12a were identical in all aspects with those obtained in experiment 3.8.1.

3.8.3. 9,11β-dichloro-7α-(methoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (13, Scheme 4)

A solution of (7α,17α)-9(11)-ene-7 (2.5 g, 6.27 mmol) in anhydrous pyridine (5.1 mL, 62.7 mmol) and chlorobenzene (25 mL) was cooled to 0 °C. SO₂Cl₂ (1.0 mL, 12.54 mmol) was added dropwise and the mixture was stirred for 30 min at 0 °C. The cooling bath was removed and the stirring was continued for 1 h at room temperature. The reaction mixture was diluted with H₂O (100 mL) and the product was extracted with CH₂Cl₂ (3 × 50 mL).

Scheme 4. Synthesis of the (7α,11β,17α)-9,11-dichloro derivative 13.

The combined organic extracts were washed with aqueous saturated NaHCO₃ solution (150 mL) followed by H₂O (150 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give a brown foam. The crude product was purified by column chromatography over silica
gel with 5–30% AcOEt/CH₂Cl₂ gradient elution to afford 9,11-dichloro impurity 13 (1.21 g, 41% yield) as white crystals. Rᵣ = 0.27 for 15% AcOEt/CH₂Cl₂. M.p. 230–231 °C. |ₓ|D = +123.30 (c 1.0, CHCl₃). FT-IR (pelets, KBr) ν (cm⁻¹): 3020, 2987, 2949, 2901, 2880, 1765, 1717, 1657, 1616, 1424, 1361, 1343, 1243, 1213, 1187, 1159, 1036, 1014, 965, 916, 868, 777, 665, 641, 601, 511. HRMS (ESI): calcd. for C₂₄H₃₀O₅NaCl₂ [M + Na⁺] 491.13625, found 491.1386. ¹H- and ¹³C-NMR spectra, see Table 7.

3.8.4. 3-Oxo-17α-pregn-4-ene-7α,9:21,17-dicarbolactone (14, Imp. A, Scheme 5)

A suspension of 11-hydroxyester (7α,11α,17α)-12 (5.0 g, 12.0 mmol) in anhydrous pyridine (25 mL) was cooled to 5 °C and MeCl (1.1 mL, 14.4 mmol) was added dropwise. A mixture was stirred at room temperature for 2 h followed by pyridine evaporation under reduced pressure. The residue was suspended in CH₂Cl₂ (25 mL), washed subsequently with aqueous 1 M HCl solution (3 × 25 mL), H₂O (25 mL) and brine (25 mL). The organic phase was dried over anhydrous Na₂SO₄, filtered and evaporated under reduced pressure.

The crude product was purified by column chromatography over silica gel with 0.5–2% MeOH/CH₂Cl₂ gradient elution to afford mesylate (5.04 g, 85% yield) as white crystals. A mixture of mesylate (2.5 g, 5.05 mmol), AcONa (4.14 g, 50.5 mmol), 80% AcOH (15 mL) and H₂O (5 mL) was heated at 70 °C for 3 h followed by AcOH evaporation under reduced pressure. Saturated aqueous Na₂CO₃ solution (25 mL) was added to the residue and the product was extracted with CH₂Cl₂ (3 × 25 mL). The (7α,17α)-9(11)-enester 7 (13.58 g, 71% yield). The combined organic phases were washed with brine (100 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel with 5–30% AcOEt/CH₂Cl₂ gradient elution to afford 7α,9:21,17-dicarbolactone 14 (1.61 g, 83% yield) as a white solid. M.p. 204–206 °C (lit. 205–206 °C [17]). |ₓ|D = −2.41 (c 1.0, CHCl₃). FT-IR (pelets, KBr) ν (cm⁻¹): 2964, 2875, 1770, 1674, 1627, 1452, 1383, 1344, 1268, 1196, 1177, 1157, 1110, 1047, 971, 914, 874, 814, 722, 657, 593, 509. HRMS (ESI): calcd. for C₂₅H₂₉O₅Na [M + Na⁺] 407.18289, found 407.1847. ¹H- and ¹³C-NMR spectra, see Table 7.

3.8.5. 7α-(Methoxycarbonyl)-3-oxo-17α-pregn-4,9(11)-diene-21,17-carbolactone (7, Imp. C, Scheme 6)

Imidazole (13.08 g, 192.08 mmol) was added to a solution of 11-hydroxyester (7α,11α,17α)-12 (20.0 g, 48.02 mmol) in THF (150 mL) and cooled to −10 °C. SO₂Cl₂ (8.2 mL, 100.84 mmol) was added dropwise and the mixture was stirred for 30 min. at −10 °C. The cooling bath was then removed and the stirring was continued for 1 h at room temperature. The reaction mixture was diluted with H₂O (100 mL) and the product was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic extracts were washed with aqueous saturated NaHCO₃ solution (100 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give a yellowish white foam.

The crude product was recrystallized from ethanol and a mixture of dichloromethane/diethyl ether to give (7α,17α)-9(11)-enester 7 (13.58 g, 71% yield). Rᵣ = 0.17 for 15% AcOEt/CH₂Cl₂. M.p. 204–206 °C (lit. 205–206 °C [17]). |ₓ|D = +2.75 (c 1.0, CHCl₃). FTIR (KBr) ν (cm⁻¹): 3052, 2967, 2908, 2875, 1774, 1732, 1666, 1622, 1463, 1438, 1421, 1378, 1332, 1268, 1166, 1090, 1012, 966, 920, 868, 834, 770, 665, 614, 531, 475. HRMS (ESI): calcd. for C₂₅H₃₀O₅Na [M + Na⁺] 421.19855, found 421.1986. ¹H- and ¹³C-NMR spectra, see Table 4.
3.8.6. 7β-(methoxycarbonyl)-3-oxo-17α-pregna-4,9(11)-dione-21,17-carbolactone (7a, Schemes 7 and 8)

Method 1

The (7α,17α)-9(11)-enester 7 (3.2 g, 8.03 mmol) was added to a suspension of MeONa (1.52 g, 28.11 mmol) in anhydrous MeOH (20 mL) and then refluxed for 24 h. The mixture was cooled to room temperature and aqueous 3 M HCl solution (100 mL) was added. The product was extracted with CH₂Cl₂ (3 × 25 mL). The combined organic phases were dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give a brownish foam.

The crude mixture of products was purified by column chromatography over silica gel with 5–30% AcOEt/CH₂Cl₂ gradient elution to give (7α,17α)-9(11)-enester 7a (1.18 g, 37% yield) as a white solid, a mixture of epimers 7 and 7a (0.78 g) and (7α,17α)-9(11)-enester 7 (1.12 g).

(7β,17α)-9(11)-enester 7a: R₁ = 0.34 for 15% AcOEt/CH₂Cl₂. M.p. 167–168 °C. [α]$_{D}^{20}$ = +0.50 (c 1.0, CHCl₃). FT-IR (pellets, KBr) ν (cm⁻¹): 3053, 2970, 2883, 2847, 1773, 1731, 1671, 1618, 1435, 1369, 1299, 1265, 1188, 1159, 1090, 1017, 990, 910, 868, 816, 792, 769, 665, 602, 521, 480. HRMS (ESI): calcd. for C₂₄H₃₀O₅Na [M + Na]⁺ 421.19855, found 421.2006. ¹H- and ¹³C-NMR spectra, see Table 4. The characterization data of 7 were identical in all aspects with those obtained in experiment 3.8.5.

Method 2

Imidazole (1.31 g, 19.20 mmol) was added to a solution of 11-hydroxyester (7β,11α,17α)-12a (2.0 g, 4.80 mmol) in THF (25 mL) and cooled to −20 °C. SO₂Cl₂ (0.8 mL, 10.08 mmol) was added dropwise and the mixture was stirred for 30 min. at −20 °C. The cooling bath was then removed and the stirring was continued for 1 h at room temperature. The reaction mixture was diluted with H₂O (10 mL) and the product was extracted with CH₂Cl₂ (3 × 25 mL).

The combined organic extracts were washed with aqueous saturated NaHCO₃ solution (50 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give a yellowish white foam. The crude product was purified by column chromatography over silica gel with 5–20% AcOEt/CH₂Cl₂ gradient elution to give (7β,17α)-9(11)-enester 7a (1.62 g, 85% yield). The characterization data of 7a were identical in all aspects with those obtained in Method 1.
3.8.7. \(\alpha\)-(Methoxycarbonyl)-3-oxo-17\(\alpha\)-pregna-4,11(12)-diene-21,17-carbolactone (7b)

The recrystallization mother liquors of 7 (9.81 g) were chromatographed on silica gel using varying mixtures of ethyl acetate, dichloromethane, acetonitrile and toluene. Early cuts of the 5–30% AcOEt/CH2Cl2 gradient elution afforded (7\(\beta\),17\(\alpha\))-9(11)-enester 7a (0.48 g) and (7\(\alpha\),17\(\alpha\))-11(12)-enester 7b (0.73 g) as white crystalline products. An analytical sample of 7b was prepared by a recrystallization from ethanol. Succeeding cuts of 5–30% AcOEt/CH2Cl2 gradient elution gave (7\(\alpha\),11\(\beta\),17\(\alpha\))-9,11-dichloro impurity 14 (0.84 g), 7a,21,17-dilactone 13 (1.16 g) and (7\(\alpha\),17\(\alpha\))-9(11)-enester 7 (2.75 g) as white crystalline products. Finally, crystalline 11-hydroxyesters (7\(\beta\),11\(\alpha\),17\(\alpha\))-12a (0.17 g) and (7\(\alpha\),11\(\alpha\),17\(\alpha\))-12 (0.33 g) were obtained on further gradient elution with 10–50% MeCN/PhCH3. (7\(\alpha\),17\(\alpha\))-11(12)-enester 7b: \(R_f = 0.29\) for 15% AcOEt/CH2Cl2. M.p. 232–234 °C. \([\alpha]_{D}^{20} = +33.42\) (c 1.0, CHCl3). FT-IR (pellets, KBr) \(\nu\) (cm\(^{-1}\)): 3028, 2971, 2949, 2878, 1775, 1723, 1664, 1616, 1465, 1438, 1380, 1334, 1267, 1195, 1172, 1048, 1013, 918, 868, 788, 716, 669, 525, 454. HRMS (ESI): calcd. for C\(_{24}\)H\(_{30}\)O\(_3\)Na [M + Na]\(^+\) 421.1991, found 421.1988. \(^{1}\)H- and \(^{13}\)C-NMR spectra, see Table 4.

The characterization data of 7, 7a, 12, 12a, 13 and 14 were identical in all aspects with those obtained in experiments 3.8.1–3.8.6, respectively.

3.8.8. 11\(\alpha\),12\(\alpha\)-Epoxypreg-4-ene-21,17-carbolactone (2b, Imp. B, Scheme 9)

2,2,2-Trichloroacetamide (0.52 g, 3.17 mmol) and Na\(_2\)HPO\(_4\) (0.35 g, 2.48 mmol) were added to a stirred solution of (7\(\alpha\),17\(\alpha\))-11(12)-enester 7b (0.55 g, 1.38 mmol) in CH\(_2\)Cl\(_2\) (15 mL). The mixture was cooled to 15 °C and H\(_2\)O\(_2\) (35%, 8.4 mL) was added dropwise. After being stirred at room temperature for 48 h, the mixture was diluted with H\(_2\)O (15 mL) and the product was extracted with CH\(_2\)Cl\(_2\) (3 × 10 mL). The combined organic extracts were washed in succession with 3% aqueous Na\(_2\)SO\(_4\) solution (25 mL), 1 M aqueous NaOH solution (20 mL), 1 M aqueous HCl solution (20 mL) and brine (25 mL).

The organic phase was dried over anhydrous Na\(_2\)SO\(_4\), filtered and concentrated under reduced pressure to give a yellowish white foam. The crude 11,12-epoxide was purified by column chromatography over silica gel with 1–10% Me\(_2\)CO/CH\(_2\)Cl\(_2\) gradient elution to give 2b (0.34 g, 60% yield) as a white solid. \(R_f = 0.37\) for 10% Me\(_2\)CO/CH\(_2\)Cl\(_2\). M.p. 209–210 °C. \([\alpha]_{D}^{20} = +24.63\) (c 1.0, CH\(_2\)Cl\(_2\)). FT-IR (pellets, KBr) \(\nu\) (cm\(^{-1}\)): 3531, 3430, 3024, 2952, 2880, 1780, 1725, 1664, 1614, 1463, 1437, 1421, 1404, 1381, 1335, 1295, 1268, 1237, 1194, 1174, 1072, 1034, 1011, 977, 962, 920, 880, 836, 784, 664,
629, 605, 524, 497, 454. HRMS (ESI): calcd. for C_{24}H_{30}O_{6}Na [M + Na]^{+} 437.1940, found 437.1942. \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectra, see Table 8.

3.8.9. 9,11\-Epoxy-7\-\((methoxycarbonyl)\)-3-oxo-17\-pregn-4-ene-21,17-carbolactone (2\(a\), Imp. E, Schemes 10 and 11).

Method 1

Eplerenone (3.1 g, 7.48 mmol) was added to a suspension of MeONa (1.41 g, 26.17 mmol) in anhydrous MeOH (25 mL) and then refluxed for 24 h. The mixture was cooled to room temperature and 3 M aqueous HCl solution (150 mL) was added. The product was extracted with CH\(_2\)Cl\(_2\) (3 × 25 mL).

The characterization data of \(\alpha\)-\(2\) were identical in all aspects with those obtained in Method 1.

Method 2

2,2,2-Trichloroacetamide (0.98 g, 6.02 mmol) and Na\(_2\)HPO\(_4\) (1.21 g, 8.53 mmol) were added to a stirred solution of \((7\beta,11\alpha,17\alpha)-11(12)\)-enester \(7a\) (2.0 g, 5.02 mmol) in CH\(_2\)Cl\(_2\) (50 mL). The mixture was cooled to 15 °C and H\(_2\)O\(_2\) (35%, 4.2 mL) was added dropwise. After being stirred at room temperature for 48 h, the mixture was diluted with H\(_2\)O (20 mL) and the product was extracted with CH\(_2\)Cl\(_2\) (3 × 25 mL). The combined organic extracts were washed in succession with 3% aqueous Na\(_2\)SO\(_4\) solution (25 mL), 1 M aqueous NaOH solution (20 mL), 1 M aqueous HCl solution (20 mL) and brine (25 mL).

The characterization data of \(\alpha\)-\(2\) were identical in all aspects with those obtained in experiment 3.8.10.

The combined organic phases were dried over anhydrous Na\(_2\)SO\(_4\), filtered and concentrated under reduced pressure to give a yellowish white solid. The crude mixture of epimers \(\alpha\)-\(2\) and \(\alpha\)-\(2\) was purified by column chromatography over silica gel with 1–10% Me\(_2\)CO/CH\(_2\)Cl\(_2\) gradient elution to give the \(\alpha\)-\(2\) (1.52 g, 49% yield) as a white solid, a mixture of \(\alpha\)-\(2\) and \(\alpha\)-\(2\) (0.55 g) and \(\alpha\)-\(2\),\(\alpha\)-\(2\)-9,11-epoxyester \(2\) (0.77 g).

\((7\beta,11\alpha,17\alpha)-9,11\)-epoxyester \(2\) \(\alpha\)-\(2b\): \(R\_f\) = 0.43 for 10% Me\(_2\)CO/CH\(_2\)Cl\(_2\). M.p. 249–251 °C (lit. 254–258 °C [9]). \(\alpha\)_D = +12.53 (c 1.0, CHCl\(_3\)). FT-IR (pellets, KBr) \(\nu\) (cm\(^{-1}\)): 3030, 2972, 2953, 2929, 2883, 1774, 1734, 1674, 1619, 1436, 1384, 1296, 1269, 1195, 1165, 1080, 1013, 905, 866, 797, 659, 576, 521, 414. HRMS (ESI): calcd. for C\(_{24}\)H\(_{31}\)O\(_6\) [M + H]\(^{+}\) 415.21152, found 415.2113. \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectra, see Table 8.

The characterization data of \(\alpha\)-\(2\) were identical in all aspects with those obtained in experiment 3.8.10.

The organic phase was dried over anhydrous Na\(_2\)SO\(_4\), filtered and concentrated under reduced pressure to give a yellowish white foam. The crude product was purified by column chromatography
over silica gel with 1–10% Me₂CO/CH₂Cl₂ gradient elution to give the (7β,11α,17α)-9,11-epoxyester 2a (1.44 g, 69% yield) as a white solid. The characterization data of 2a were identical in all aspects with those obtained in Method 1.

3.8.10. 9,11α-Epoxo-7α-(methoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (eplerenone, 2) (without purification of the enester 7, Scheme 12)

Imidazole (6.53 g, 96.04 mmol) was added to a solution of 11-hydroxyester (7α,11α,17α)-12 (10.0 g, 24.01 mmol) in THF (100 mL) and cooled to −10 °C. SO₂Cl₂ (4.1 mL, 50.42 mmol) was added dropwise and the mixture was stirred for 30 min. at −10 °C. The cooling bath was then removed and the stirring was continued for 1 h at room temperature.

The reaction mixture was diluted with H₂O (100 mL) and the product was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic extracts were washed with aqueous saturated NaHCO₃ and the mixture was cooled to 15 °C and H₂O₂ (35%, 4.2 mL) was added dropwise. After being stirred at room temperature for 18 h, H₂O (50 mL) and crushed ice (50 g) were added to the reaction mixture and the layers were separated. The organic phase was washed with NaOH (0.5 M, 100 mL), HCl (0.5 N, 100 mL), brine (100 mL), dried over anhydrous Na₂SO₄ and filtered under reduced pressure to give a yellowish white foam. The crude product was recrystallized twice from ethanol and from 2-butanone to give the pharmaceutical grade (7α,11α,17α)-9,11-epoxyester 2 (5.20 g, 52% yield) as a white crystals. Rₖ = 0.27 for 10% Me₂CO/CH₂Cl₂. M.p. 241–243 °C (lit. 240–242 °C [17]). [α]D²⁰ = +1.55 (c 1.0, CHCl₃). FTIR (pellets, KBr) ν (cm⁻¹): 2997, 2969, 2950, 2877, 1778, 1725, 1656, 1619, 1460, 1444, 1427, 1382, 1293, 1273, 1183, 1160, 1081, 1018, 985, 919, 848, 797, 722, 694, 656, 543, 520, 460. HRMS (ESI): calcd. for C₂₃H₃₀O₆Na [M + Na]+ 437.1940, found 437.1941. ¹H- and ¹³C-NMR spectra, see Table 8. The recrystallization mother liquors of 2 (4.1 g) were chromatographed on silica gel using varying mixtures of acetone and dichloromethane. Early cuts of the 1–10% Me₂CO/CH₂Cl₂ gradient elution afforded the (7α,11β,17α)-9,11-dichloro impurity 13 (0.46 g) and the (7β,11α,17α)-9,11-epoxy ester 2a (0.19 g). Succeeding cuts of the 1–10% Me₂CO/CH₂Cl₂ gradient elution gave the 7,9:21,17-dilactone 14 (0.85 g) and the (7α,11α,17α)-11,12-epoxy ester 2b (0.26 g). The characterization data of 2a, 2b, 13 and 14 were identical in all aspects with those obtained in experiments 3.8.3, 3.8.4, 3.8.8 and 3.8.9, respectively.

4. Conclusions

Two new process-related impurities of the antihypertensive drug eplerenone (2) were synthesized and fully characterized. The impurities were identified as 11α-hydroxy-7β-(methoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (12a) and 9,11β-dichloro-7α-(methoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (13). Additionally, seven other eplerenone impurities poorly described in the literature, including four impurities A, B, C and E listed in the European Pharmacopoeia 8.4, were isolated and/or synthesized and fully characterized by
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IR, NMR, HRMS/ESI and SCXRD. All the impurities resulted from side reactions taking place on the steroid rings B and C of the starting (7α,11α,17α)-11-hydroxyester 12 and the key intermediate (7α,17α)-9(11)-enester 7, including epimerization of the C-7 asymmetric center, oxidation, dehydration, chlorination and lactonization. The full identification and characterization of the impurities should be useful for the quality control and the validation of the analytical methods in the manufacture of eplerenone.

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**Sample Availability:** Samples of the compounds 2, 2a–b, 7, 7a–b, 12, 12a, 13 and 14 are available from the authors.