Cinnamoyl Derivatives from Cordia Platythyrsa and Chemiotaxonomical Value of the Cordia Genus

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Abstract: Phytochemical investigation of the roots and stem barks of Cordia platythyrsa (Boraginaceae) had led to the isolation of two new cinnamates, the cordicinnamate A compound 1 and the cordicinnamate B compound 2 along with four known compounds. Their structures were established by spectroscopic analysis mainly FAB and TOF –MS, ¹H NMR and ¹³C NMR, COSY, HMBC, HSQC and by comparison with literature data. The cinnamoyl derivatives were reported for the first time in the Cordia genus. The isolation and identification of cinnamoyl derivatives in Cordia genus improve the chemiotaxonomy value in this genus.

Keywords: Boraginaceae, Cordia Platythyrsa, Cinnamoyl Derivatives, Chemiotaxonomy

1. Introduction

The genus cordia belongs to the family of boraginaceae and it is found in warm regions [1]. The decoction of several species of cordia genus has been used in traditional medicine to treat influenza, fever, pneumonia, coughs, insomnia, stomach-ache, parasitic [2] and infections [3]. Previous phytochemical investigations of cordia genus led to the isolation of pyrrolizidine alkaloids, terpenoids flavonoids, lignans, meroterpenoids naphtoquinones [4]. In addition, phytochemical studies of Cordia platythyrsa have revealed the presence of shingolipids, Cordiachromes A-F [5] and xanthones [6]. Its leaves are used for the treatment of convulsions and sleeping sickness (maceration) [7]. With a view to extending the phytochemical investigations of these species, the present paper report the isolation and structure characterization of two new cinnamates namely cordicinnamate A-B and other known compounds.

2. Experimental

2.1. General Experimental Procedure

Melting points were determined on a Büchi 434 melting point apparatus and were uncorrected. ¹H NMR (400 MHz) and ¹³C NMR (150MHz) spectra were recorded at room temperature in CDCl₃ using a Bruker AVANCE AM 400 and AMX 500 NMR instruments. Chemical shifts are given in δ (ppm) value relative to TMS as internal standard. ESI-TOF mass spectra operating in positive mode were recorded on a finnigan MAT 312 and FAB mass spectra was recorded on Jeol JMS HX 110 mass spectrometer. Silica gel (230-400 meshes) and sephadex gel (LH-20) were used for Column Chromatography (CC) and vacuum liquid chromatography (VLC). Thin Layer Chromatography (TLC) was performed on precoated silica gel plates (60 F254, Macherey-Nagel) using the system solvent n-hexane – EtOAc (9.2:0.8, 8:2) and EtOAc-MeOH (6.5:3.5) as eluent. Spots were visualized by UV light (λmax = 254 nm, 366 nm) and were observed after using sulphuric acid (50%) as spraying reagent.
2.2. Plant Material

The stem barks and roots of *Cordia platythyrsa* were collected in Yaoundé town (Cameroon) and were identified by Dr N. Tsabang, of the Centre for Study of Medicinal Plants of Yaoundé (Cameroon). One voucher specimen (N°43625/HNC) was deposited at the National Herbarium of Cameroon (NHC).

2.3. Extraction and Isolation

The powdered roots (2 Kg) were extracted with CH$_2$Cl$_2$-MeOH (1:1) at room temperature for 72 hours. The resulting extract was evaporated to dryness under reduced pressure to yield a dark residue (12.72 g). This residue was partitioned with n-hexane, EtOAc and MeOH. The EtOAc extract was evaporated under reduced pressure to afford 8 g of residue. This residue was submitted to vacuum liquid chromatography (VLC) on silica gel 230-400 meshes eluted with n-hexane – EtOAc and EtOAc in order of increasing polarity. As result, 7 fractions (B0, B1, B2, B3, B4, B5 and B6) were collected on the basis of TLC. Fraction B3 and B4 were subjected to CC purification using silica gel 230-400 meshes eluted with n-hexane – EtOAc (8:2) to give compounds 1 (2mg) and 2 (2mg). Fraction B6 was subjected to CC purification using silica gel 230-400 meshes eluted with EtOAc- MeOH (6:5:3:5) afforded to 4 (20mg).

The powdered stem barks (6.5 Kg) were extracted with CH$_2$Cl$_2$-MeOH (1:1) at room temperature for 72 hours. The resulting extract was evaporated to dryness under reduced pressure to yield a dark residue (200 g). This residue was partitioned with n-hexane, EtOAc and MeOH. The EtOAc extract was evaporated under reduced pressure to give 97 g of residue which was in turn submitted to vacuum liquid chromatography (VLC) on using silica gel 230-400 meshes eluted with n-hexane – EtOAc and EtOAc- MeOH in order of increasing polarity. As result, 3 fractions (S1, S2 and S3) were collected on the basis of TLC. Fraction S3 was subjected to CC using silica gel 230-400 meshes eluted with n-hexane – EtOAc (9:2:0:8) afforded to 9 subfractions. Subfraction S375 was purified using sephadex gel LH-20 eluted with MeOH to afford compounds 1 (1.90 mg) and 5 (2 mg).

The powdered stem barks (6.5 Kg) were extracted with CH$_2$Cl$_2$-MeOH (1:1) at room temperature for 72 hours. The resulting extract was evaporated to dryness under reduced pressure to yield a dark residue (12.72 g). This residue was partitioned with n-hexane, EtOAc and MeOH. The EtOAc extract was evaporated under reduced pressure to afford 8 g of residue. This residue was submitted to vacuum liquid chromatography (VLC) on silica gel 230-400 meshes eluted with n-hexane – EtOAc and EtOAc- MeOH in order of increasing polarity. As result, 3 fractions (S1, S2 and S3) were collected on the basis of TLC. Fraction S3 was subjected to CC using silica gel 230-400 meshes eluted with n-hexane – EtOAc (9:2:0:8) afforded to 9 subfractions. Subfraction S375 was purified using sephadex gel LH-20 eluted with MeOH to afford compounds 1 (1.90 mg) and 5 (2 mg).

The powdered roots (2 Kg) were extracted with CH$_2$Cl$_2$-MeOH (1:1) at room temperature for 72 hours. The resulting extract was evaporated to dryness under reduced pressure to yield a dark residue (200 g). This residue was partitioned with n-hexane, EtOAc and MeOH. The EtOAc extract was evaporated under reduced pressure to give 97 g of residue which was in turn submitted to vacuum liquid chromatography (VLC) on using silica gel 230-400 meshes eluted with n-hexane – EtOAc and EtOAc- MeOH in order of increasing polarity. As result, 7 fractions (B0, B1, B2, B3, B4, B5 and B6) were collected on the basis of TLC. Fraction B3 and B4 were subjected to CC purification using silica gel 230-400 meshes eluted with n-hexane – EtOAc (8:2) to give compounds 1 (2mg), 3 (2mg) and 6 (3mg). Fraction B6 was subjected to CC purification using silica gel 230-400 meshes eluted with EtOAc- MeOH (6:5:3:5) afforded to 4 (20mg).

3. Results and Discussion

3.1. Identification of the Compounds

Compound 1 was obtained as a white powder. Its molecular formula was deduced as C$_{28}$H$_{46}$O$_3$ by negative mode FAB-MS at m/z = 429.0 [M-H]$^-$. Comparison of the NMR data (table 1) of compound 2 with those of compound 1 indicated a slight modification in the cinnamoyl moiety of compound 2. Instead of the aromatic proton at C-3' and methyl group at C-6', signals detected were those of an aliphatic chain and aromatic proton at δ$_{H}$ 1.64 (2H, m, H-1'''), 1.18 (36H, S, H-5''-21'''), 1.53 (3H, m, H-22'''), 3.53 (2H, t, H-1''''), 3.32 (3H, S, 23'''-OCH$_3$) and 1.53 (3H, S, 6'-CH$_3$). Proton signals owing to aliphatic chain were observed at δ$_{H}$ 1.18(12H, H, H-5'-'-14''), 4.11(2H, t, H-1'''') and 0.81(6H, S, 2x4''-CH$_3$). The $^1$C NMR spectrum with signals at δ$_{C}$ 168.0 (C, C-1), 114.7(CH,C-2''), 123.1 (CH,C-3''), 109.2 (CH,C-5''), δ 115.7 (CH,C-2') and 144.0 (CH, C-3) had demonstrated the presence of cinnamoyl moiety [8, 9]. Aliphatic chain was identified and characterized by signals at δ 22.7, 31.9, 23.0 (10 CH$_{3}$, C-5''-'-14'''), 37.2 (C, C-4''). Protons position of aliphatic chain and cinnamoyl moiety were supported by COSY correlations (figure 1) H-2'/ H-3, H-2''/H-3'' and H-1''/H-2''. The carbon signals were assigned by HSQC spectrum and were confirmed by HMBC spectrum. Using HMBC correlations (figure 1) of H-3''''/C-4''''; 4''''-OCH$_3$/C-4'''', 6''''-CH$_3$/C-6'''' and H-1''''/C-1'', C-2'' had permitted to link aliphatic chain, methoxy and methyl group to cinnamoyl moiety. From the spectral evidences and literature data, the structure of compound 1 was characterized as 1-(4''''-,4''''-dimethylpentadecyl)-4''''-methoxy-6''''-methyl cinnamate (Cordicinnamate A).

Compound 2 isolated as a white powder, exhibited molecular formula C$_{30}$H$_{48}$O$_3$ as determined from its positive mode TOF-MS at m/z = 603.5003 [M+3H]$^+$. Comparison of the NMR data (table 1) of compound 2 with those of compound 1 indicated a slight modification in the cinnamoyl moiety of compound 2. Instead of the aromatic proton at C-3'' and methyl group at C-6''', signals detected were those of an aliphatic chain and aromatic proton at δ$_{H}$ 1.64 (2H, m, H-1'''''), 1.18 (36H, S, H-3'''''-21'''''), 1.53 (2H, m, H-22''''''), 3.53 (2H, t, H-1''''''), 7.03(1H, d, J=8 Hz, H-2''), 1.32 (2H, m, H-4'''''') attributed to a second aliphatic chain. Protons positions of the above aliphatic chain and cinnamoyl moiety were confirmed by COSY correlations (figure 2) H-2'/H-3, H-5'''/H-6'', H-1''''/H-2'' and H-22'''''/H-23''''. The HMBC correlation (figure 2) of H-5'''/C-1', C-3 and H-1''''/C-2'', C-4'''' demonstrated the presence of ester group [8, 9]. Thus the structure of 2 was established as 3''''-(23'''''-methoxy triecicosanyl)-1-(pentyl)-4''''-methoxy cinnamate (Cordicinnamate B).
Compound 3 was obtained as a white powder. Its molecular formula was assigned as C$_{15}$H$_{20}$O$_5$ as deduced from TOF-MS (positive mode) m/z = 282 [M+2H]$^+$. Comparison of the NMR data (table 1) of compound 3 with those of compound 2 indicated the signal at δ$_H$ 3.91 (1H, S, 3'-OCH$_3$), 5.81(1H, S, 4'-OH) and 3.61(2H, t) instead of aliphatic chain, methoxy and methylene group respectively in $^1$HNMR spectrum. Its structure (Figure 3) was elucidated by 1D and 2D NMR spectroscopy and it was identified as 1-(5''-hydroxypentyl)-4-hydroxy-3-methoxycinnamate [10]. Structures of the compounds 4-6 were elucidated by 1D and 2D NMR spectroscopy. All the physical and spectral data were identical to the reported values in literature [6, 11-14]. The compounds were identified as methyl orsellinate 4, paramethoxytoylehexanoate 5 and 3-O-$\beta$-D-glucopyranosyl-$\beta$-sitosterol 6 (Figure 3).

Previous studies of Cordia species led to various skeletons of compounds. The triterpenoids have been isolated both from Cordia verbenacea DC and Cordia multisipicata [15] and Dammarane-type triterpenes both from Cordia spinescens and Cordia multisipicata [16]. The Meroterpenoid naphthoquinones have been isolated both from the roots of Cordia linncae and Cordia corymbosa [17]. The roots of Cordia curassavica [18] and wood of Cordia fragrantissima afforded hydroquinones [19]. The magnesium lithospermate, Calcium rosmarinate and magnesium rosmarinate were isolated from the leaves of Cordia spinescens [20]. The investigation of the heartwood of Cordia millenii yielded terpenoid quinones [21]. Hydroquinone terpenoids were isolated both from Cordia alliodora and the heartwood of Cordia elaeagnoides [22]. The terpenoid glycosides have been isolated from Cordia oblique [23].

In addition, phytochemical studies on Cordia platytherys yielded to two new cinnamoyl derivatives, Cordicinnamate A (compound 1) and Cordicinnamate B (compound 2). To the best of our knowledge this is the first time that cinnamates have been reported from Cordia genus.

3.2. Cordicinnamate A (Compound 1)

White powder; MP =116-118°C; $^1$H NMR (CDCl$_3$, 400MHz) and $^{13}$NMR (CDCl$_3$, 150MHz) data see table 1; FAB-MS m/z = 429.0 [M-H]$^-$ (calcd. for C$_{28}$H$_{46}$O$_5$, 430.0).
Table 1. 1H NMR (400MHz) and 13C NMR (150MHz) data for compounds 1-3 in CDCl3.

| Position | δC | δH[mult, J (Hz)] | δC | δH[mult, J (Hz)] | δC | δH[mult, J (Hz)] |
|----------|----|-----------------|----|-----------------|----|-----------------|
| 1        | 168.0 | - | 168.0 | - | 167.0 | - |
| 2        | 115.7 | 6.21 (1H, d, J =16Hz) | 115.0 | 6.21 (1H, d, J =16Hz) | 117.0 | 6.27 (1H, d, J =15.5Hz) |
| 3        | 144.0 | 7.53 (1H, d, J =16Hz) | 144.0 | 7.57 (1H, d, J =16Hz) | 144.0 | 7.58 (1H, d, J =15.5Hz) |
| 1''      | 127.0 | - | 127 | - | 127.0 | - |
| 2''      | 114.7 | 6.81 (1H, d, J =8Hz) | 109.0 | 7.00 (1H, d, J =1.5Hz) | 109.0 | 7.01 (1H, d, J =1.6Hz) |
| 3        | 123.1 | 6.99 (1H, d, J =8Hz) | 148 | - | 146.0 | - |
| 4''      | 147.0 | - | 147.0 | - | 115.0 | - |
| 5''      | 109.2 | 6.97 (1H, S, 1H) | 114.0 | 6.76 (1H, d, J =8Hz) | 123.0 | 7.05 (1H, d, J =8Hz) |
| 6''      | 135.0 | - | 125.5 | 6.03 (1H, d, J =8Hz) | 116.0 | 6.86 (1H, d, J =1.6Hz, 8Hz) |
| 3'-OCH3 | - | - | - | - | 56.0 | 3.91 (1H, S) |
| 4'-OH   | - | - | - | - | 5.81 (3H, S) |
| 6'-CH3  | 55.9 | 3.85(3H, S) | 56.0 | 3.85(3H, S) | - | - |
| 1''      | 64.6 | 4.11 (1H, t) | 64.6 | 4.11 (2H, t) | 64.5 | 4.16 (2H, t) |
| 2''      | 26.0 | 1.60(2H, m) | 29.0 | 1.48(2H, m) | 30.0 | 1.6(2H, m) |
| 3''      | 28.0 | 0.86(2H, m) | 23.0 | 1.28(2H, m) | 26.0 | 1.2-1.5(2H, m) |
| 4''      | 37.2 | - | 26.0 | 1.32(2H, m) | 25.9 | 1.5(2H, m) |
| 5''      | 55.9 | 3.85(3H, S) | 56.0 | 3.85(3H, S) | - | - |
| 6''      | 56.0 | 3.85(3H, S) | - | - | - | - |
| 1''      | 14.0 | 0.81(3H, t) | 64.0 | 3.61(2H, m) | - | - |
| 5''-14'' | 22.7 | 23.0, 31.9 | 0.71-0.86(12H,m) | - | - | - |
| 15''     | 14.2 | 0.78(3H, m) | - | - | - | - |
| 4''-CH3  | 19.4 | 0.75(3H, m) | - | - | - | - |
| 4''-CH3  | 18.8 | 0.75(3H, m) | - | - | - | - |
| 1''      | - | - | 34.5 | 2.20(2H, t) | - | - |
| 2''      | - | - | 28.0 | 1.64(2H, m) | - | - |
| 3''-21''' | - | - | 23.0 | 1.18(36H, S) | - | - |
| 22'''    | - | - | 25.0 | 1.53(2H, m) | - | - |
| 23'''    | - | - | 62.5 | 3.53(2H, t) | - | - |
| 23''''-OCH3 | - | - | 49.5 | 3.32(3H, S) | - | - |

3.3. Cordicinnamate B (Compound 2)

White powder; MP =185°C; 1H NMR (CDCl3, 400MHz) and 13C NMR (CDCl3, 150MHz) data see table 1; TOF-MS m/z = 603.5003 [M+3H] (calcd. for C39H68O4, 600.5003).

3.4. 1-(5''-hydroxypentyl)-4-hydroxy-3-methoxy Cinnamate (Compound 3)

White powder; MP = 63-64°C; 1H NMR (CDCl3, 400MHz) and 13C NMR (CDCl3, 150MHz) data see table 1; TOF-MS m/z= 282 [M+2H] (calcd. for C15H20O5, 280.0).

4. Conclusion

Phytochemical studies on Cordia platythyrsa yielded two cinnamoyl derivatives isolated for the first time on Cordia genus along with five others known compounds. To the best of our knowledge, this is the first time to isolate the cinnamoyl skeleton on the Cordia genus. The isolation and the identification of cinnamoyl derivatives in cordia genus improve the chemiotaxonomy value of the Cordia genus. Further studies aiming to isolate and identify compounds for the other fractions are needed.

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