SYNTHESIS OF $O$-ALKYL DERIVATIVES OF DEHYDROZINGERONE ANALOGUES

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ABSTRACT. Vanillin and isobutyl methyl ketone (4-methylpentan-2-one) reacts under Claisen–Schmidt conditions yielding corresponding dehydrozingerone analogue, (E)-1-(4-hydroxy-3-methoxyphenyl)-5-methylhex-1-en-3-one. A small series of its $O$-alkyl derivatives was prepared by alkylation of free phenolic group with corresponding alkyl halides. Products had been tested for their biological activity and demonstrated relatively strong in vitro antimicrobial activity towards different strains of bacteria and fungi. All new compounds were well characterized by IR, $^1$H and $^{13}$C NMR spectroscopy and physical data.

Keywords: vanillin, dehydrozingerone, enone system, microbiological activity.

INTRODUCTION

Chalcones, 1,3-diaryl-2-propen-1-ones, are one of the important classes of organic compounds, which have a unique chemical structure with conjugated double bonds and a completely delocalized π-electron system on both aromatic rings.

Chalcones and its derivatives exhibit different pharmacological and biological activities. They show good antifungal [1-3], antimicrobial [4-6], anticonvulsant [7], antioxidant [8-10], antiprotential [11], antitrichomoniasis [12] antimalarial [13-15], anti-inflammatory [16-18], trypsin inhibitors [19] and anti-cancer activity [20-23]. At this kind of molecules is important to identify the fragment of their structure responsible for previously described activities. It has been reported that free phenolic group in ring at position 4- was key factor important for potent antibacterial activity of licochalcone A and licochalcone B [24-25]. Activity is dependant on the nature, position and number of substituent on aromatic rings.

From ginger root, fresh or dried, many different kinds of compounds, such as dehidrozingerone, zingerone, gingerol, shogaol, paradol and their derivatives have been isolated. Vanillin fragment is presented in all kinds of those compounds. Those compounds also have well expressed bioactivity, such as anticancer, anti-oxidant, antimicrobial, anti-inflammatory, antidiabetic, anti-allergic [26-28].
Starting from this fact we supposed that vanillin is suitable substrate for further transformation, due its easy modification, by O-alkylation [29-30], by coupling reactions and forming of divanillin [31], formylations in position 5- [32].

Dehydrozingerone, 4-(4-hydroxy-3-methoxyphenyl)-3-buteno-2-one, Fig. 1, one of pungent constituents of ginger rhizome, also exhibits a wide range of biological activities [33-35]. Although conjugate enone system is presented in this phenolic compound, its structure differs from chalcones; instead of the aryl group to the carbonyl is connected the methyl one. Enone system could be easily transformed into some usable heterocyclic derivatives [36-38].

In continuation of our interest in synthesis of vanillin derivatives we synthesized, starting from vanillin, dehydrozingerone analogue (E)-1-(4-hydroxy-3-methoxyphenyl)-5-methylhex-1-en-3-one 2a. Starting from this enone compound several O-alkyl derivatives were synthesized, and all new products were characterized by their spectral data (IR, $^1$H NMR and $^{13}$C NMR). Their biological activity toward some strains of microorganisms wave been tested.

MATERIALS AND METHODS

General remarks

All starting chemicals were commercially available and used as received, except that the solvents were purified by distillation. Chromatographic separations were carried out using silica gel 60 (Merck, 230-400 mesh ASTM) whereas silica gel on Al plates, layer thickness 0.2 mm (Merck), was used for TLC. IR spectra were recorded on a Perkin-Elmer One FT-IR spectrometer with a KBr disc, $\nu$ in cm$^{-1}$; NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer (200 MHz for $^1$H and 50 MHz for $^{13}$C), using CDCl$_3$ as solvent and TMS as the internal standard. $^1$H and $^{13}$C NMR chemical shifts were reported in parts per million (ppm) and were referenced to the solvent peak; CDCl$_3$ (7.26 ppm for $^1$H and 76.90 ppm for $^{13}$C). Multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Coupling constants ($J$) are in Hertz (Hz).

The antimicrobial activity was estimated by determination of the minimal inhibitory concentration (MIC) using the broth microdilution method against five species of bacteria and five species of fungi.

Experimental procedure

1. Chemistry

Vanillin and 4-methylpentan-2-one reacts under Claisen-Schmidt conditions yielding corresponding enone compound (E)-1-(4-hydroxy-3-methoxyphenyl)-5-methylhex-1-en-3-one, 2a in good yield, Scheme 1. Compound 2a was prepared according to slightly modified procedure [39]. A set of its O-alkyl derivatives, compounds 2b-g, was prepared by alkylation of free phenolic group in 2a with corresponding alkyl halides, according to the described literature procedures, [29,30,40], Scheme 2.
Compounds 2a and 2b are known compounds and their chemical synthesis was published earlier [41,42]. Compounds 2c-g are new compounds and their structure and spectral data are given.

Scheme 1. Synthesis of (E)-1-(4-hydroxy-3-methoxyphenyl)-5-methylhex-1-en-3-one, 2a

Scheme 2. Synthesis of (E)-1-(4-alkoxy-3-methoxyphenyl)-5-methylhex-1-en-3-one, 2b-g

2. Chemical synthesis

2.1. Synthesis of (E)-1-(4-hydroxy-3-methoxyphenyl)-5-methylhex-1-en-3-one, 2a

Vanillin, 4.56 g (30 mmol) was dissolved in 50 mL of methanol and 100 mL of 4-methylpentan-2-one was added. To a well stirred homogenous mixture 10% NaOH (25 mL) was added and mixture was stirred for 48 hours at 60°C. Solvents, methanol and methyl-i-buthyl ketone, was removed under reduced pressure and to oily residue 100 mL of water was added, then acidified with 2M HCl (pH=2). Product was extracted with CH$_2$Cl$_2$, 3×50 mL, and organic layer was washed with water and dried with anhydrous Na$_2$SO$_4$. Solvent was distilled off and residue was distilled with steam until no more methyl-i-buthyl ketone odour was presented in distillate. Water/oil residue were extracted with toluene, 3×50 mL, organic phase was dried and solvent was evaporated under reduced pressure, yielding a yellow oily product (E)-1-(4-hydroxy-3-methoxyphenyl)-5-methylhex-1-en-3-one, 2a which crystalize on standing.

Yield: 89.6%; m.p. 82°C; IR (KBr): 2970, 2952, 2870, 1678, 1601, 1584, 1517, 1286, 1268, 1146, 1065, 988 cm$^{-1}$; $^1$HNMR (CDCl$_3$): 0.98 (d, 6H, $J=6.6$Hz, 2xCH$_3$), 2.16-2.30 (m, 1H, CH), 2.53 (d, 2H, $J=7.0$Hz, CH$_2$), 3.92 (s, 3H, OCH$_3$), 6.25 (s, 1H, OH), 6.61 (d, 1H, $J=16.2$Hz, CH), 6.93 (d, 1H, $J=8.2$Hz, Ar-H), 7.05-7.11 (m, 2H, Ar-H), 7.48 (d, 1H, $J=16.2$Hz, CH); $^{13}$C NMR (CDCl$_3$): 22.6, 25.3, 49.6, 55.9, 109.5, 114.8, 123.3, 124.4, 127, 142.7, 146.9, 148.2, 200.4 (CO).

2.2. General procedure for synthesis of (E)-1-(4-alkoxy-3-methoxyphenyl)-5-methylhex-1-en-3-one, 2b-g
A mixture of (E)-1-(4-hydroxy-3-methoxyphenyl)-5-methylhex-1-en-3-one, 2a (0.470 g, 2 mmol), corresponding alkyl halide (excess, 10 mmol) and K₂CO₃ (1.94 g, anhydrous, 14 mmol) in acetone (50 mL) was heated to reflux overnight under argon. Acetone and excess of alkyl halide was evaporated under reduced pressure, solid residue was dissolved in water and extracted with CH₂Cl₂ (3x50 mL). The combined extracts were washed with water and dried over anhydrous Na₂SO₄. After removal of the main part of solvent the residue was filtered over short SiO₂ column and then distilled with steam, if necessary. Products, compounds 2c and 2d were isolated as oils, and others crystallize on standing.

2.2.1. Synthesis of (E)-1-(3,4-dimethoxyphenyl)-5-methylhex-1-en-3-one, 2b

CH₃I, 1.45 g (excess, 10 mmol); m.p. 67°C; Yield: 99.4%.

IR (KBr): 2957, 2926, 2869, 1683, 1648, 1595, 1582, 1517, 1464, 1366, 1275, 1190, 1141, 1017, 975 cm⁻¹; ¹H NMR (CDCl₃): 0.98 (d, 6H, J=6.6Hz, 2xCH₃), 2.17-2.31 (m, 1H, CH), 2.53 (d, 2H, J=6.8Hz, CH₂), 3.92 (d, 6H, J=1.2Hz, 2xOCH₃), 6.63 (d, 1H, J=16.2Hz, CH), 6.88 (d, 1H, J=8.2Hz, Ar-H), 7.08-7.16 (m, 2H, Ar-H), 7.49 (d, 1H, J=16.0Hz, CH); ¹³C NMR (CDCl₃): 22.6, 25.2, 49.6, 55.9, 109.7, 111.1, 122.8, 124.7, 127.5, 142.3, 149.2, 151.2, 200.1 (CO).

2.2.3. Synthesis of (E)-1-(4-ethoxy-3-methoxyphenyl)-5-methylhex-1-en-3-one, 2c

C₂H₅I, 1.56 g (excess, 10 mmol); oil; Yield: 80.9%.

IR (KBr): 2957, 2871, 1683, 1649, 1596, 1513, 1466, 1266, 1232, 1141, 1035, 983 cm⁻¹; ¹H NMR (CDCl₃): 0.98 (d, 6H, J=6.6Hz, 2xCH₃), 1.49 (t, 3H, J=7.0Hz, CH₃), 2.17-2.30 (m, 1H, CH), 2.53 (d, 2H, J=7.0Hz, CH₂), 3.91 (s, 3H, OCH₃), 4.14 (q, 2H, J=7.0Hz, CH₂), 6.62 (d, 1H, J=16.2Hz, CH), 6.86 (d, 1H, J=8.2Hz, Ar-H), 7.08-7.14 (m, 2H, Ar-H), 7.49 (d, 1H, J=16.0Hz, CH); ¹³C NMR (CDCl₃): 14.6, 22.7, 25.3, 49.7, 55.9, 64.4, 110.1, 112.3, 122.8, 124.6, 127.3, 142.5, 149.5, 150.7, 200.2 (CO).

2.2.4. Synthesis of (E)-1-(3-methoxy-4-isopropoxyphenyl)-5-methylhex-1-en-3-one, 2d

i-C₃H₇I, 1.70 g (excess, 10 mmol); oil; Yield: 77.7%.

IR (KBr): 2960, 2871, 1682, 1652, 1595, 1509, 1466, 1420, 1268, 1230, 1138, 1110, 983 cm⁻¹; ¹H NMR (CDCl₃): 0.98 (d, 6H, J=6.6Hz, 2xCH₃), 1.39 (d, 6H, J=6.0Hz, 2xCH₃), 2.17-2.30 (m, 1H, CH), 2.53 (d, 2H, J=7.0Hz, CH₂), 3.91 (s, 3H, OCH₃), 4.14 (q, 2H, J=6.8Hz, CH₂), 6.62 (d, 1H, J=16.0Hz, CH), 6.88 (d, 1H, J=8.0Hz, Ar-H), 7.07-7.14 (m, 2H, Ar-H), 7.49 (d, 1H, J=16.2Hz, CH); ¹³C NMR (CDCl₃): 10.3, 22.3, 22.7, 25.3, 49.7, 55.9, 71.3, 110.7, 114.6, 122.7, 124.6, 127.5, 142.5, 149.8, 150.3, 200.2 (CO).

2.2.5. Synthesis of (E)-1-(3-methoxy-4-propoxyphenyl)-5-methylhex-1-en-3-one, 2e

n-C₃H₇Br, 1.23 g (excess, 10 mmol); m.p. 49-50°C; Yield: 91.7%.

IR (KBr): 2960, 2939, 2874, 1689, 1645, 1619, 1595, 1515, 1466, 1423, 1271, 1226, 1140, 1033, 973 cm⁻¹; ¹H NMR (CDCl₃): 0.98 (d, 6H, J=6.6Hz, 2xCH₃), 1.05 (t, 3H, J=7.6Hz, CH₃), 1.79-1.98 (m, 2H, CH₂), 2.17-2.30 (m, 1H, CH), 2.53 (d, 2H, J=6.8Hz, CH₂), 3.91 (s, 3H, OCH₃), 4.02 (t, 2H, J=6.8Hz, CH₂), 6.62 (d, 1H, J=16.0Hz, CH), 6.87 (d, 1H, J=8.2Hz, Ar-H), 7.07-7.14 (m, 2H, Ar-H), 7.49 (d, 1H, J=16.2Hz, CH); ¹³C NMR (CDCl₃): 10.3, 22.3, 22.7, 25.3, 49.7, 56, 70.5, 110.3, 112.5, 122.9, 124.6, 127.3, 142.5, 149.6, 150.9, 200.2 (CO).
2.2.6. Synthesis of (E)-1-(4-butoxy-3-methoxyphenyl)-5-methylhex-1-en-3-one, 2f

\[ n-C_7H_9Br, 1.37 \text{ g (excess, 10 mmol); m.p. 54°C; Yield: 96.5%}. \]

**IR (KBr):** 2954, 2871, 1651, 1622, 1598, 1514, 1466, 1425, 1273, 1169, 1140, 1042, 981 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)): 0.98 (dt, 9H, \(J=6.6\text{Hz}, 2\times\text{CH}_3\)), 1.41-1.59 (m, 2H, CH\(_2\)), 1.77-1.92 (m, 2H, CH\(_2\)), 2.18-2.30 (m, 1H, CH), 2.53 (d, 2H, \(J=7.0\text{Hz}, \text{CH}_2\)), 3.90 (s, 3H, OCH\(_3\)), 4.05 (t, 2H, \(J=6.8\text{Hz}, \text{CH}_2\)), 6.62 (d, 1H, \(J=16.0\text{Hz}, \text{CH}\)), 6.87 (d, 1H, \(J=8.0\text{Hz}, \text{Ar-H}\)), 7.07-7.14 (m, 2H, Ar-H), 7.49 (d, 1H, \(J=16.0\text{Hz}, \text{CH}\)); \(^{13}\)C NMR (CDCl\(_3\)): 13.8, 19.1, 22.7, 25.3, 31, 49.7, 55.9, 68.7, 110.3, 112.4, 122.9, 124.6, 127.3, 142.5, 149.6, 150.9, 200.2 (CO).

2.2.7. Synthesis of (E)-1-(4-benzyloxy-3-methoxyphenyl)-5-methylhex-1-en-3-one, 2g

\[ C_6H_5CH_2Cl, 1.27 \text{ g (excess, 10 mmol); m.p. 71-72°C; Yield: 81.3%}. \]

**IR (KBr):** 2956, 2930, 2862, 1679, 1646, 1624, 1595, 1512, 1465, 1455, 1256, 1163, 1138, 1030, 981 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)): 0.97 (d, 6H, \(J=6.6\text{Hz}, 2\times\text{CH}_3\)), 2.16-2.29 (m, 1H, CH), 2.52 (d, 2H, \(J=6.8\text{Hz}, \text{CH}_2\)), 3.92 (s, 3H, OCH\(_3\)), 5.18 (s, 2H, CH\(_2\)), 6.61 (d, 1H, \(J=16.2\text{Hz}, \text{CH}\)), 6.87 (d, 1H, \(J=8.0\text{Hz}, \text{Ar-H}\)), 7.03-7.09 (m, 2H, Ar-H), 7.26-7.51 (m, 6H, CH, Ar-H); \(^{13}\)C NMR (CDCl\(_3\)): 22.7, 25.3, 49.7, 55.9, 70.8, 110.4, 113.5, 122.6, 124.8, 127.1, 127.8, 127.9, 128.5, 136.5, 142.3, 149.8, 150.4, 200.2 (CO).

3. Antimicrobial activity

**Microorganisms and media**

The following bacteria were used as test organisms in this study: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *B. cereus* (ATCC 10987), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). All of the bacteria used were obtained from the American Type Culture Collection (ATCC). The bacterial cultures were maintained on Müller-Hinton agar substrates (Torlak, Belgrade). The fungi used as test organisms were: *Aspergillus flavus* (ATCC 9170), *A. fumigatus* (ATCC 1022), *Candida albicans* (ATCC 10259), *Penicillium purpureascens* (ATCC 48987), *P. verucosum* (ATCC 48959). All of the fungi were from the American Type Culture Collection (ATCC). The fungal cultures were maintained on potato dextrose (PD) agar, except for *C. albicans* that was maintained on Sabouraud dextrose (SD) agar (Torlak, Belgrade). All of the cultures were stored at 4°C and subcultured every 15 days.

Bacterial inoculi were obtained from bacterial cultures incubated for 24 h at 37°C on Müller-Hinton agar substrates and brought up by dilution according to the 0.5 McFarland standard to approximately \(10^8\) CFU/mL. Suspensions of fungal spores were prepared from freshly mature (3- to 7-day-old) cultures that grew at 30°C on a PD agar substrate. The spores were rinsed with sterile distilled water, used to determine turbidity spectrophotometrically at 530 nm, and were then further diluted to approximately \(10^6\) CFU/mL according to the procedure recommended by NCCLS (1998).

**Minimal inhibitory concentration (MIC)**

The minimal inhibitory concentration (MIC) was determined by the broth microdilution method using 96-well micro-titer plates [43]. A series of dilutions with
concentrations ranging from 20 to 0.004 mg/mL of the tested compounds was used in the experiment against every microorganism tested. The starting solutions of tested compounds was obtained by measuring off a certain quantity of the compounds and dissolving it in 5% dimethyl sulphoxide (DMSO). Two-fold dilutions of the compounds were prepared in a Müller-Hinton broth for bacterial cultures and a SD broth for fungal cultures. The MIC was determined with resazurin. Resazurin is an redox indicator used for the evaluation of microbial growth. It is a blue non-fluorescent dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells. The boundary dilution without any changing color of resazurin was defined as the MIC for the tested microorganism at a given concentration. As a positive control of growth inhibition, streptomycin and ketoconazole was used. A 5% DMSO solution was used as a negative control for the influence of the solvents.

RESULTS AND DISCUSSION

Dehydrozingerone analogues 2a, with i-buthyl group attached to carbonyl, were synthesized under Claisen–Schmidt conditions yielding corresponding enone compound (E)-1-(4-hydroxy-3-methoxyphenyl)-5-methylhex-1-en-3-one, 2a in good yield. This compound reacts with various alkyl halides yielding corresponding O-alkyl derivatives 2b-g. Synthesized compounds 2a-g was well characterized by spectral data and microbiological activity. The tested compounds 2a-c demonstrated relatively strong antimicrobial activity inhibiting all tested microorganisms. The MIC for these compounds relative to the tested microorganisms ranged from 0.009 to 5 mg/mL. The strongest antibacterial activity was found in 2a component, which inhibited all the species of bacteria, especially B. subtilis where measured MIC value was extremely low (0.009 mg/mL). This compound also inhibited the growth of the tested fungi but in slightly higher concentrations (MIC values were from 0.312 to 0.625 mg/mL). Compound 2d inhibited only B. subtilis and B. cereus. Other tested components (2e-g) did not inhibit any of the test microorganisms. Among the bacteria, the highest resistance was shown in E. coli, while the most sensitive was B. subtilis. Among the fungi, the most sensitive appeared to be C. albicans.

The antimicrobial activity was compared with the standard antibiotics, streptomycin (for bacteria) and ketoconazole (for fungi). The results showed that standard antibiotics had stronger activity than tested samples as shown in Table 1. In these experiments, the compounds examined at the same concentrations showed a slightly stronger antibacterial than antifungal activity. These results could be expected due to the fact that numerous tests proved that bacteria are more sensitive to the antibiotic compared to fungi [44]. The reason for different sensitivities between fungi and bacteria can be found in different permeabilities of the cell wall. The cell wall of the gram-positive bacteria consists of peptidoglycans (murein) and teichoic acids, while the cell wall of gram-negative bacteria consists of lipopolysaccharides and lipopoliproteins [45], whereas, the cell wall of fungi consists of polysaccharides such as chitin and glucan [46].

Compounds 2b-c have substituent with short carbon chain on oxygen (Me and Et), whereas compounds 2d-g have longer carbon chain on oxygen (i-Pr, n-Pr, n-Bu and Bz). We suppose that structure of alkyl group is responsible for the lack of their activity.

From this point, the results of this study suggest that dehydrozingerone analogue derivatives 2a-g are promising candidates, after some modification, for testing of some other activities.
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| Microorganisms | Staphylococcus aureus | Bacillus subtilis | Bacillus cereus | Escherichia coli | Proteus mirabilis | Aspergillus flavus | Aspergillus fumigatus | Candida albicans | Penicillium purpurescens | Penicillium verrucosum |
|----------------|----------------------|------------------|----------------|-----------------|------------------|------------------|----------------------|-------------------|---------------------------|---------------------------|
| Tested compounds |                     |                  |                |                 |                  |                  |                      |                   |                           |                           |
| 2a              | 0.156               | 0.009            | 0.019          | 0.312           | 0.156            | 0.625            | 0.312                | 0.312             | 0.312                     | 0.312                     |
| 2b              | 0.625               | 0.078            | 0.156          | 2.5             | 1.25             | 5                | 2.5                  | 1.25              | 5                          | 5                          |
| 2c              | 1.25                | 0.156            | 0.312          | 2.5             | 1.25             | 5                | 5                    | 1.25              | 5                          | 5                          |
| 2d              | -                   | 0.625            | 1.25           | -               | -                | -                | -                    | -                 | -                          | -                          |
| 2e              | -                   | -                | -              | -               | -                | -                | -                    | -                 | -                          | -                          |
| 2f              | -                   | -                | -              | -               | -                | -                | -                    | -                 | -                          | -                          |
| 2g              | -                   | -                | -              | -               | -                | -                | -                    | -                 | -                          | -                          |
| Antibiotics     | 0.031               | 0.016            | 0.016          | 0.062           | 0.062            | 0.078            | 0.078                | 0.039             | 0.156                     | 0.156                     |

Values given as mg/mL
Antibiotics: Streptomycin (for bacteria) and Ketoconazole (for fungi),