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AN ISOCOUMARIN AND OTHER PHENOLIC COMPONENTS OF *ONONIS NATRIX*

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Key Word Index—*Ononis natrix*; Leguminosae; Trifolieae; dihydroisocoumarin; resorcinol derivatives; homopterocarpin.

Abstract—From the *n*-hexane extract of the whole aerial part of flowering *Ononis natrix*, besides homopterocarpin and some known terpenoids and sterols, three new compounds: 8-hydroxy-6-methoxy-3-undecyl-3,4-dihydroisocoumarin, 5-(2-acetoxytridecyl)-3-methoxyphenol and 5-(2-hydroxytridecyl)-3-methoxyphenol have been isolated.

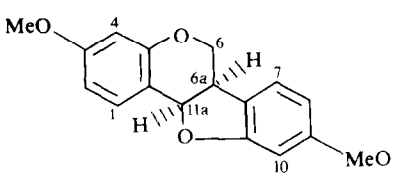
INTRODUCTION

Ononis natrix (Leguminosae, tribe Trifolieae) is a small plant known for its medicinal properties which are similar to those of *O. spinosa*. The infusion of its roots has diuretic and antirheumatic properties and has been used for the treatment of certain disturbances of the urinary tract [1]. Although there are no reports on the chemical composition of *O. natrix*, some studies on *O. spinosa* and *O. arvensis* have been published [2–8].

RESULTS AND DISCUSSION

From the methanol-soluble fraction of the *n*-hexane extract of *O. natrix*, the well-known compounds phytol (**1**), betulaprenol-8 (**2**), sitosterol (**3**), stigmasta-7,24(28)-dien-3β-ol (**4**) and 24-methylenecycloartanol (**5**), as well as the common metabolite of species of the subfamily Lotoideae, homopterocarpin (**6**), were isolated. Compounds **1** and **3** were identified by direct comparison with authentic samples and **2** and **4**–**6** through comparison of their physical and spectroscopic properties with those reported for these substances [9–12]. Furthermore, three new natural polyketides were also isolated.

The first substance, in the chromatographic elution order, shows mp 90° (MeOH), M^+ at *m/z* 348 ($C_{21}H_{32}O_4$) and IR absorptions of carbonyl group (1665 cm^{-1}), benzene ring and aliphatic and aromatic C–O bonds. The observation of an intramolecular hydrogen bond in its IR spectrum, besides the positive reaction with ferric chloride and the bathochromic shift of 30 nm induced by aluminium chloride on the 302 nm UV absorption maximum, indicated the presence of a phenolic hydroxyl adjacent to the carbonyl (similar shifts 35–55 nm have been described for Band I of 5-hydro-



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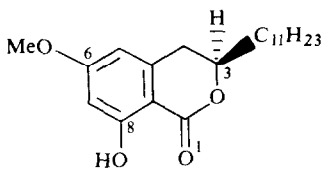

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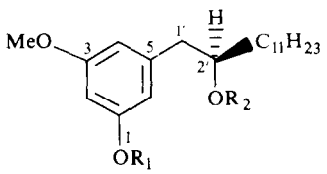

8 $R_1 = H$, $R_2 = Ac$
9 $R_1 = R_2 = Ac$
10 $R_1 = R_2 = H$

xyflavonoids [13]). The ^{1}H NMR showed signals due to two aromatic protons in the *meta* position (δ 6.36 and 6.24, *d*, J = 2.5 Hz) and of a benzylic methylene (2.84, *br d*, J = 7 Hz), which is coupled with the proton (4.46, *m*) of a methine group bearing an oxygenated function, and slightly coupled with the aromatic proton at 6.24. Moreover, signals of an aromatic methoxy group (δ 3.80) and of a linear aliphatic chain (1.27) with its terminal methyl group (0.87, *t*, J = 6.5 Hz) were also observed.

These data indicated structure **7** for this compound [14–16] which also agreed with the fragmentation pattern of the mass spectrum (see Experimental). The assignment of the absolute configuration at C-3 as *R* was based on the negative value of its $[\alpha]_D$, which is similar to those of known related compounds [17], and on the negative Cotton effect shown at 269 nm [18, 19]. Thus, this substance is (3*R*)-8-hydroxy-6-methoxy-3-undecyl-3,4-dihydroisocoumarin (**7**).

The second substance is an oil whose mass spectrum shows M^+ at *m/z* 364 ($C_{22}H_{36}O_4$) and whose IR spectrum shows absorption of phenyl, phenolic hydroxyl and acetate groups. Its ^{1}H NMR shows signals of three aromatic protons (δ 6.31 *br s*) and of a benzylic methylene (2.74, *d*, J = 6.5 Hz), which is coupled with the proton (5.01, *m*) of a methine group bearing an acetate function. Furthermore, two methyl singlets (δ 2.00 and 3.77) and the absorptions of a linear chain (1.24, *br s* and 0.87, *t*) are also observed. On acetylation, a new oily product with M^+ at *m/z* 406 ($C_{24}H_{38}O_5$) and whose IR and ^{1}H NMR spectra showed the expected changes, was obtained. Lithium aluminium hydride reduction of the natural hydroxyacetate and of its acetylation product gave the same compound, whose IR spectrum showed hydroxyl absorptions but no carbonyl absorption and whose ^{1}H NMR spectrum principally differs from the starting compounds in the multiplet at δ 3.70 (5.01 for the hydroxyacetate and 5.02 for its acetate). The above data, as well as the UV absorptions between 270 and 280 nm [20, 21] and the peaks observed in the MS (see Experimental), agree with the structure of 5-(2-acetoxytridecyl)-3-methoxyphenol (**8**) for this substance, and the structures **9** and **10** for its acetylation and reduction products.

The third substance, mp 70°, is identical to **10**, the reduction product of **8** and **9**. Thus, its structure corresponds to 5-(2-hydroxytridecyl)-3-methoxyphenol. The *R* configuration at C-2′ of **8**–**10** was established according to the negative sign of its $[\alpha]_D$, similar to those other compounds with one chiral center similarly substituted [22], and this is in agreement with the stereochemistry at the same carbon atom of the related compound, **7**.

The co-occurrence of **7**, **8** and **10** in this plant, and that of 8-hydroxy-3-undecyl-3,4-dihydroisocoumarin and 3-tridecylphenol in the alga *Caulocystis cephalornitos* [23], could signify that both types of compounds, 3-alkyldihydroisocoumarins and 3-alkylphenols (or 5-alkylresorcinols), have a common biogenetic precursor in a structure related to either orsellinic or olivetonic acids.

EXPERIMENTAL

Mps are uncorr. UV spectra were recorded in EtOH and IR spectra in $CHCl_3$. ^{1}H NMR spectra were recorded at 60 MHz, in $CDCl_3$, using TMS as int. standard. Specific rotations were taken in $CHCl_3$. CD curves were recorded in EtOH. EIMS were obtained at 70 eV. Analytical TLC was performed on Si gel G (Merck 7331), prep. TLC on Si gel $PF_{254-366}$ (Merck 7748) and CC on Si gel (Merck 7734).

Flowering plant material was collected in July at Aldealengua (Salamanca, Spain). Air-dried material (1.72 kg) was extracted in a Soxhlet with *n*-hexane (9 l.) yielding, after cooling for 20 hr at 4°, 48.80 g (2.83% of the dry wt of the plant) of soluble hexane extract. After successive separation of the MeOH-insoluble fraction, of those components giving clathrates with satd methanolic urea and of the acidic compounds, by 4% aq. NaOH extraction, 5.28 g (8% of the extract) of a neutral part was obtained. The components were isolated by Si gel (250 g) CC and purified by prep. TLC and/or crystallization.

From the less polar fractions, after saponification, **1** (90 mg) and **2** (48 mg) were isolated. From the remaining fractions, **6** (123 mg), **7** (70 mg), **1** (78 mg), **5** (80 mg, mp 122°, lit. [11] 121–122°), **3** (500 mg), **4** (100 mg mp 147°, lit [10] 146–149°), **8** (40 mg) and **10** (36 mg) were obtained.

Homopterocarpin (**6**). Eluted with *n*-hexane–Et_2O (49:1) by Si gel CC. Mp 88° (MeOH). EIMS *m/z*: 348 [M^+] ($C_{17}H_{16}O_4$).

$$[\alpha]_\lambda = \frac{\begin{matrix}589 & 578 & 546 & 436 & 365\end{matrix}}{\begin{matrix}-152.1 & -159.7 & -181.8 & -310.6 & -452.9\end{matrix}} \quad (c\ 0.34).$$

IR ν_{max} cm^{-1}: 1625, 1500, 1290, 1160, 1150, 1120, 1035, 950, 840. ^{1}H NMR: δ 3.40–3.65 (2H, *m*, H-6a and H-6′), 3.75 (3H, *s*, –OMe) and 3.78 (3H, *s*, –OMe), 4.22 (1H, *dd*, J = 8 and 3.5 Hz, H-6), 5.49 (1H, *d*, J = 6.5 Hz, H-11a), 6.35–6.55 (3H, *m*, H-4, H-8 and H-10), 6.62 (1H, *dd*, J = 9 and 3 Hz, H-2) 7.13 (1H, *d*, J = 9 Hz, H-1), 7.42 (1H, *d*, J = 9 Hz, H-7). UV λ_{max}^{EtOH} nm (log ε): 282 (3.69), 287 (3.61), 309 (3.45).

8-*Hydroxy*-6-*methoxy*-3-*undecyl*-3,4-*dihydroisocoumarin* (**7**). Eluted with *n*-hexane–Et_2O (49:1) by Si gel CC. Mp 98° (MeOH).

$$[\alpha]_\lambda = \frac{\begin{matrix}589 & 578 & 546 & 436 & 365\end{matrix}}{\begin{matrix}-23.2 & -23.5 & -26.1 & -47.1 & -71.3\end{matrix}} \quad (c\ 0.31).$$

IR ν_{max} cm^{-1}: 1665, 1630, 1600, 1500, 1245, 1160, 1115, 915; 845. ^{1}H NMR: δ 0.87 (3H, *t*, J = 6 Hz, H-11′), 1.27 (20H, *m*, H-1′–H-10′), 2.84 (2H, *d*, J = 7 Hz, H-4), 3.80 (3H, *s*, –OMe), 4.46 (1H, *m*, H-3), 6.24 (1H, *d*, J = 2.5 Hz, H-5), 6.36 (1H, *d*, J = 2.5 Hz, H-7). UV λ_{max}^{EtOH} nm (log ε): 302 (4), 266 (4.46), 214 (4.60). EIMS *m/z* (rel. int.): 348 [M^+] (11), 330 (2), 193 (8), 165 (18), 164 (25), 43 (100). CD: $\Delta\varepsilon_{302} = +0.18$, $\Delta\varepsilon_{269} = -0.90$, $\Delta\varepsilon_{249} = +0.26$, $\Delta\varepsilon_{232} = -0.98$.

5-(2′-*Acetoxytridecyl*)-3-*methoxyphenol* (**8**). Eluted with *n*-hexane–Et_2O (4:1) by Si gel CC. Oil.

$$[\alpha]_\lambda = \frac{\begin{matrix}589 & 578 & 546 & 436 & 365\end{matrix}}{\begin{matrix}-0.7 & -0.7 & -1.2 & -2.3 & -22.6\end{matrix}} \quad (c\ 1.24).$$

IR ν_{max} cm^{-1}: 3610, 1730, 1605, 1500, 1260, 1210, 1115, 840. ^{1}H NMR: δ 0.87 (3H, *t*, J = 6 Hz, H-13′), 1.24 (20H, *m*, H-3′–H-12′), 2.00 (3H, *s*, –OAc), 2.74 (2H, *d*, J = 6.5 Hz, H-1′), 3.77 (3H, *s*, –OMe), 5.01 (1H, *m*, H-2′), 6.31 (3H, *br s*, H-2, H-4 and H-6). UV λ_{max}^{EtOH} nm (log ε): 281 (4.12), 274 (4.15). EIMS *m/z* (rel. int.): 364 [M]$^+$ (2), 304 [M – HOAc]$^+$ (2), 262 [M – HOAc – CH_2CO)]$^+$ (2), 138 (100), 137 (32). Acetate (**9**): oil.

$$[\alpha]_\lambda = \frac{\begin{matrix}589 & 578 & 546 & 436 & 365\end{matrix}}{\begin{matrix}0.0 & 0.0 & 0.0 & -0.7 & -1.7\end{matrix}} \quad (c\ 0.86).$$

IR ν_{max} cm^{-1}: 1775, 1745, 1615, 1600, 1250, 1210, 1135, 1075, 1025, 830. ^{1}H NMR: δ 0.88 (3H, *t*, J = 6 Hz, H-13′), 1.26 (20H, *m*, H-3′–H-12′), 2.00 (3H, *s*, aliphatic –OAc), 2.26 (3H, *s*, aromatic –OAc), 2.80 (2H, *d*, J = 6.5 Hz, H-1′) 3.77 (3H, *s*, –OMe), 5.02 (1H, *m*, J = 6.5 Hz, H-2′), 6.58 (3H, *m*, H-2, H-4 and H-6).

UV λ_{max}^{EtOH} nm (log ε): 278 (3.05), 273 (3.07). Reduction of **8** with $LiAlH_4$ (in Et_2O) gave **10**, mp 70° (*n*-hexane–CH_2Cl_2).

$$[\alpha]_\lambda = \frac{\begin{matrix}589 & 578 & 546 & 436 & 365\end{matrix}}{\begin{matrix}-0.6 & -0.6 & -1.3 & -2.0 & -21.8\end{matrix}} (c\ 1.20).$$

IR ν_{max} cm^{-1}: 3610, 1605, 1500, 1150, 1065, 853. ^1H NMR: δ 0.89 (3H, *t*, *J* = 6.5 Hz, H-13′), 1.27 (20H, *m*, H-3′–H-12′), 2.68 (2H, *m*, H-1′), 3.70 (1H, *m*, H-2′), 3.74 (3H, *s*, –OMe), 6.30 (3H, *br s*, H-2, H-4 and H-6). UV λ_{max}^{EtOH} nm (log ε): 281 (3.86), 274 (3.89).

5-(2′-*Hydroxytridecyl*)-3-*methoxyphenol* (**10**) Isolated by prep. TLC from the more polar chromatographic fraction, eluted with *n*-hexane–Et_2O (1:1) by Si gel CC, identical to the $LiAlH_4$ reduction product of **8**. Diacetate **9** identical to that of **8**.

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