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# $N$-$\Delta^{13}$-DOCOSENOYLANTHRANILIC ACID AND ALKYLRESORCINOLS FROM *ONONIS NATRIX* SUBSP. *HISPANICA*

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**Key Word Index**—*Ononis natrix* subsp *hispanica*; Leguminosae, aerial parts, alkylresorcinols; $N$-$\Delta^{13}$-docosenoylanthranilic acid, (2′*R*)-6-(2-acetoxytridecyl)-2-hydroxy-4-methoxybenzoic acid

**Abstract**—$N$-$\Delta^{13}$-Docosenoylanthranilic acid (2′*R*)-6-(2-acetoxytridecyl)-2-hydroxy-4-methoxybenzoic acid, nine resorcinol derivatives, eugenol and the flavone nevadensin were isolated from the acidic fraction of the hexane extract of *Ononis natrix* subsp. *hispanica*.

## INTRODUCTION

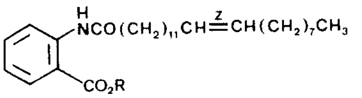
The presence of resorcinol derivatives in *Ononis speciosa* Lag [1] and in *O. natrix* L subsp *natrix* [2, 3] suggests that these substances are characteristic of the genus *Ononis*. This fact, added to their antibiotic and molluscicidal activities [4, 5], led us to study other Andalucian species of the genus. Here we report the results of the chemical analysis of the subspecies *hispanica*, a plant that grows in coastal zones of the Iberian Peninsula, in contrast to the subspecies *natrix*, which is typical of the interior.

## RESULTS

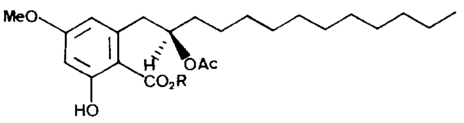
From the hexane extract of the aerial parts of *Ononis natrix* subsp. *hispanica* we isolated eugenol, the fatty acids myristic, palmitic, stearic, oleic, linoleic and linolenic, $N$-$\Delta^{13}$-docosenoylanthranilic acid (**1a**), (2′*R*)-6-(2-acetoxytridecyl)-2-hydroxy-4-methoxybenzoic acid (**2a**), the resorcinol derivatives (2′*R*)-5-(2-acetoxytridecyl)resorcinol methyl ether (**3**), (2′*R*)-5-(2-acetoxy-tridecyl)resorcinol (**4**), (2′*R*)-5-(2-hydroxytridecyl)resorcinol methyl ether (**5**), (2′*R*,8′*S*)-5-(2,8-dihydroxytridecyl)resorcinol (**6**), (2′*R*,8′*S*)-5-(2,8-dihydroxytridecyl)resorcinol methyl ether (**7**), (2′*R*)-5-(2-hydroxy-8-oxytridecyl)resorcinol (**8**), three natural isocoumarins: (3*R*)-6,8-dihydroxy-3-undecyl-3,4-dihydroisocoumarin (**9**), (3*R*)-8-hydroxy-6-methoxy-3-undecyl-3,4-dihydroisocoumarin (**10**), (3*R*,6′*S*)-6,8-dihydroxy-3(6-hydroxyundecyl)-3,4-dihydroisocoumarin (**11**) and the flavone nevadensin [6]. The structures of compounds **3**–**11** were determined by comparison of their spectroscopic data with those reported in the literature [2, 3]. Compounds **1a** and **2a** are new natural products.

The structure of **1a** was assigned through its spectroscopic properties. Its IR spectrum shows strong absorptions of *ortho* disubstituted benzene (1606, 1586, 1526, 755 $\mathrm{cm}^{-1}$), of amide group (3338, 1675, 1526, 1273 $\mathrm{cm}^{-1}$), of aromatic acid (1690, 1413 $\mathrm{cm}^{-1}$) and of *cis* disubstituted olefin (659 $\mathrm{cm}^{-1}$). In the $^1$H NMR spectrum a signal arrangement due to a system of four vicinal aromatic protons was observed (8.78 *dd*, $J_1 = 8$ Hz, $J_2 = 1.5$ Hz; 8.13 *dd*, $J_1 = 8$ Hz, $J_2 = 2$ Hz; 7 60 *ddd*, $J_1 = 8$ Hz, $J_2 = 7$ Hz, $J_3 = 2$ Hz; 7.10 *ddd*, $J_1 = 8$ Hz, $J_2 = 7$ Hz, $J_3 = 1.5$ Hz), together with the *cis* disubstituted olefin (5.33 *br t*) and the proton of secondary amide (10.98 *br s*). Its methylated derivative (**1b**) showed a similar $^1$H NMR spectrum, with the only difference being the signal due to the methyl ester of an aromatic acid group (3.93 *s*). Its CI mass spectrum showed the $[\mathrm{M}+1]^+$ at $m/z$ 472 and in the EI spectrum the basis peak appeared at $m/z$ 151, which can be interpreted as a result of a McLafferty transposition of an aromatic amide, giving place, after loss of an alkylketene, to the radical ion corresponding to the methyl anthranilate. With these data and through comparison with those reported for $N$-docosanoylanthranilic acid [7], we propose the structure $N$-docosenoylanthranilic acid for **1a**, which agrees with its $^{13}$C NMR and UV spectroscopic data. In order to establish the position of the double bond we ozonized **1a**, reduced the ozonides with sodium borohydride and, after resolution of the resulting mixture, *n*-nonanol was identified through GC by comparison with an authentical sample. Thus, the double bond was unambiguously located and therefore, **1a** is $N$-$\Delta^{13}$-docosenoylanthranilic acid.

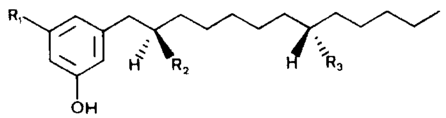
Compound **2a** was identified by means of the spectroscopic properties of its methyl derivative **2b**. This compound shows $[\mathrm{M}]^+$ at $m/z$ 422 ($C_{24}H_{38}O_6$) and IR spectrum signals due to an aromatic system (1620, 1581, 1506, 850 $\mathrm{cm}^{-1}$), acetate (1741, 1245 $\mathrm{cm}^{-1}$) and carbonyl of aromatic ester intramolecularly linked by hydrogen bonding (1658 $\mathrm{cm}^{-1}$). In its $^1$H NMR spectrum there are signals due to two aromatic protons in a *meta* relative disposition (6.35 *d*, 6.25 *d*, $J = 3$ Hz), a methoxyl group on aromatic ring (3.90 *s*) and an aromatic methyl ester (3.76 *s*). There are also signals due to a benzylic methylene (3.21 *dd*, $J_1 = 12$ Hz, $J_2 = 5$ Hz, 3.01 *dd*, $J_1 = 13$ Hz, $J_2 = 8$ Hz) which is coupled with a methine (5 10 *m*) which bears an acetoxyl function (1.93 *s*), confirmed through selective decoupling. The absorption of a linear chain (1.23 *br s* and 0.90 *br t*) is also observed. The above data led us to propose the structure (2′*R*)-6-(2-acetoxytridecyl)-2-hydroxy-4-methoxybenzoic acid for **2a**, which is in agreement with its other spectroscopic features (UV, MS and $^{13}$C NMR). In the $^{13}$C NMR spectrum the low



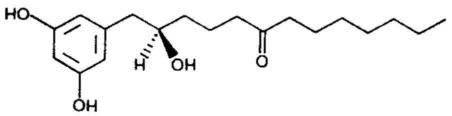
1a R=H
1b R=Me



2a R=H
2b R=Me



3 $R_1$=OMe, $R_2$=OAc, $R_3$=H
4 $R_1$=OH, $R_2$=OAc, $R_3$=H
5 $R_1$=OMe, $R_2$=OH, $R_3$=OH
6 $R_1$=OH, $R_2$=OH, $R_3$=OH
7 $R_1$=OMe, $R_2$=OH $R_3$=OH



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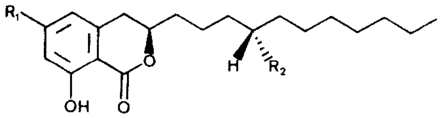

9 $R_1$=OH $R_2$=H
10 $R_1$=OMe, $R_2$=H
11 $R_1$=OH $R_2$=OH

field position of the carbons C-1′ (δ41.4) and C-3′ (δ34.5) is striking, as is the shielding of C-4′ (δ25.3) due to the presence of the acetoxyl group in C-2′. The negative sign of its $[\alpha]_D$ leads to a *R* configuration for C-2′ in agreement with that reported for other similar compounds [2, 3].

## EXPERIMENTAL

*Ononis natrix* L. subsp *hispanica* was collected in Otivar (Granada) in June, 1986. The plant was identified by Professor F Valle (Department of Botany, University of Granada) and a voucher specimen is available for inspection at the herbarium of the Faculty of Sciences of the University of Granada.

Plant material, once air-dried (3 kg) was extracted with *n*-hexane (5 l) in a Soxhlet. Solvent was vacuum removed and 64 g of extract were obtained from which the compounds were separated by their insolubility in cold MeOH and by subsequent formation of inclusion compounds with urea. By 1 M NaOH fractionation, a neutral (20 g) was sepd from an acidic (44 g) fraction. The acidic fraction (25 g) was column chromatographed over silica gel eluting with hexane–$Et_2O$–MeOH mixtures of increasing polarity.

A fraction of fatty acids was isolated (1.2 g) and GC analysed as methyl esters through a glass column (4 m length, 2 mm i.d., stationary phase SP2330 in 10% over chromosorb 100–120 mesh), initial temp. 185° during 10 min, then an increase of 3° $min^{-1}$ until 240° maintaining this temp for 20 min, injection temp 250°, detector temp. 300°. The methyl esters of the fatty acids myristic (3%), palmitic (22%), palmitoleic (1%), stearic (3%), oleic (9%), linoleic (27%), linolenic (29%) and docosenoic (2%) were identified by comparison with authentical sample. The subsequent CC fractions gave *N*-$\Delta^{13}$-docosenoylanthranilic acid (60 mg) (**1a**), (3*R*)-8-hydroxy-6-methoxy-3-undecyl-3,4-dihydroisocoumarin (90 mg) (**10**), eugenol (20 mg), (3*R*)-6,8-dihydroxy-3-undecyl-3,4-dihydroisocoumarin (90 mg) (**9**), (2′*R*)-5-(2-acetoxytridecyl)resorcinol methylether (70 mg) (**3**), (2′*R*)-5-(2-acetoxytridecyl)resorcinol (30 mg) (**4**), (2′*R*)-6-(2-acetoxytridecyl)-2-hydroxy-4-methoxybenzoic acid (40 mg) (**2a**), 5,7-dihydroxy-6,8,4′-trimethoxyflavone (nevadensin) (250 mg), (2′*R*)-5-(2-hydroxytridecyl)resorcinol methylether (40 mg) (**5**), (3*R*,6′*S*)-6,8-dihydroxy-3(6-hydroxyundecyl)-3,4-dihydroisocoumarin (220 mg) (**11**), (2′*R*,8′*S*)-5-(2,8-dihydroxytridecyl)resorcinol methylether (54 mg) (**7**), (2′*R*)-5-(2-hydroxy-8′-oxytridecyl)resorcinol (29 mg) (**8**) and (2′*R*,8′*S*)-5-(2,8-dihydroxytridecyl)resorcinol (250 mg) (**6**). All known substances were characterized by comparison of their physical and spectroscopic data with literature values [3, 6] and through transformation to their acetylated and/or methylated derivatives.

N-$\Delta^{13}$-*Docosenoylanthranilic acid* (**1a**). Oily liquid. IR $\nu_{max}^{film}$ $cm^{-1}$ 3338, 2921, 2852, 1675, 1606, 1586, 1526, 1449, 1413, 1273, 1165, 905, 802, 755. $^1$H NMR (80 MHz, $CDCl_3$) δ8.78 (1H, *dd*, $J_1$ = 8 Hz, $J_2$ = 1.5 Hz, H-3), 8.13 (1H, *dd*, $J_1$ = 8 Hz, $J_2$ = 2 Hz, H-6), 7.60 (1H, *ddd*, $J_1$ = 8 Hz, $J_2$ = 7 Hz, $J_3$ = 2 Hz, H-4), 7.10 (1H, *ddd*, $J_1$ = 8 Hz, $J_2$ = 7 Hz, $J_3$ = 1.5 Hz, H-5), 5.33 (2H, *br t*, *J* = 5 Hz, CH=CH), 2.48 (2H, *m*, CO–$CH_2$), 2.00 (4H, *m*, $CH_2$–CH=CH–$CH_2$), 1.25 (30H, *m*, $CH_2$), 0.88 (3H, *m*, Me). EIMS 70 eV, *m/z* (rel. int.) 457 $[M]^+$ (2.7), 180 (1.4), 179 (11.5), 174 (2.9), 161 (3.4), 149 (1.0), 146 (4.0), 139 (3.6), 138 (39.5), 137 (100).

*Methyl* N-$\Delta^{13}$-*docosenoylanthranilate* (**1b**). Obtained by treatment of **1a** with $CH_2N_2$ in the usual way as an oily liquid. IR $\nu_{max}^{film}$ $cm^{-1}$ 3314, 2924, 2853, 1689, 1605, 1585, 1525, 1447, 1312, 1299, 1259, 1192, 1162, 1142, 1089, 757, 702. $^1$H NMR (80 MHz CDCl$_3$): δ8.77 (1H, *br d*, $J = 8$ Hz, H-3), 8.14 (1H, *dd*, $J_1 = 8$ Hz, $J_2 = 2$ Hz, H-6), 7.60 (1H, *ddd*, $J_1 = 8$ Hz, $J_2 = 7$ Hz, $J_3 = 2$ Hz, H-4), 7.12 (1H, *br dd*, $J_1 = 8$ Hz, $J_2 = 7$ Hz, H-5), 5.35 (2H, *br t*, $J = 5$ Hz, CH=CH), 3.93 (3H, *s*, COOMe), 2.45 (2H, *m*, CH$_2$-CONH), 2.00 (4H, *m*, CH$_2$-CH=CH-CH$_2$), 1 25 (30H, *m*, CH$_2$), 0.88 (3H, *m*, −Me) $^{13}$C NMR (75 MHz CDCl$_3$) δ172.4 (*s*, CONH), 168 9 (s, COOMe), 141.8 (*s*, C-2), 134.8 (*d*, C-4), 130.9 (*d*, C-6), 130.0 (*d*, CH=CH), 122.3 (*d*, C-5), 120.4 (*d*, C-3), 114.8 (*s*, C-1), 52.4 (*q*, COOMe), 38.8 (*t*, CH$_2$CO), 32.0 (*t*, CH$_2$-CH=CH-CH$_2$), 29.9, 29.7, 29.6, 29.4, 29.3, 27.3, 25.6, 22.8 (*t*, CH$_2$), 14.2 (*q*, Me). UV $\lambda_{max}^{MeOH}$ nm (log ε): 310 (3.75), 250 (4.10). EIMS 70 eV, *m/z* (rel. int.): 472 (3), 471 [M]$^+$ (8), 440 (1), 439 (2), 230 (1), 217 (1), 216 (1), 194 (1), 193 (6), 188 (1), 175 (1), 174 (5), 162 (1), 161 (4), 154 (1), 153 (7), 152 (74), 151 (100), 147 (1), 146 (5), 137 (1), 135 (1), 133 (1), 132 (1), 123 (1), 121 (3), 120 (26), 119 (27) CIMS (CH$_4$) *m/z* (rel. int.): 473 (31), 472 [M + H]$^+$ (100), 471 (10), 470 (12)

Through ozonolysis of **1b** at −70° in MeOH followed by NaBH$_4$ reduction, we obtained a mixture whose hexane-soluble fraction contained only *n*-nonanol, which was identified by GC through comparison with an authentic sample (Capillary column, 25 m length, 0.2 mm i.d., coated with carbowax 20 M, 0 2 μm; initial temp. 50°; final temp. 200°, gradient 5° min$^{-1}$, injector 200°, detector temp. 220°).

*Methyl* (2′*R*)-6-(2-*acetoxytridecyl*)-2-*hydroxy*-4-*methoxy*benzoate (**2b**). Obtained by treatment of **2a** with CH$_2$N$_2$ Oily liquid. IR $\nu_{max}^{film}$ cm$^{-1}$: 2929, 2857, 1741, 1658, 1620, 1581, 1506, 1437, 1376, 1331, 1307, 1259, 1206, 1161, 1113, 1048, 1024, 958, 850, 835, 807, 759, 718 $^1$H NMR (80 MHz CDCl$_3$): δ11.55 (1H, *s*, OH), 6.35 (1H, *d*, $J = 3$ Hz, H-3), 6 25 (1H, *d*, $J = 3$ Hz, H-5), 5 10 (1H, *m*, H-2′), 3.90 (3H, *s*, MeO-Ar), 3.76 (3H, *s*, COOMe), 3.21 (1H, *dd*, $J_1 = 13$ Hz, $J_2 = 5$ Hz, Ar-CH), 3.01 (1H, *dd*, $J_1 = 13$ Hz, $J_2 = 8$ Hz, Ar-CH), 1.95 (3H, *s*, AcO), 1 23 (20H, *m*, CH$_2$), 0.90 (3H, *m*, −Me). $^{13}$C NMR (75 MHz, CDCl$_3$): δ171.6 (*s*, Me-COO), 170.3 (*s*, COOMe), 165 6 (*s*, C-4), 163 7 (*s*, C-2), 142.2 (*s*, C-6), 112.4 (*d*, C-5), 105.0 (*s*, C-1), 99 6 (*d*, C-3), 74.3 (*d*, C-2′), 55 3 (*q*, MeO-Ar), 51.9 (*q*, COOMe), 41.4 (*t*, C-1′), 34.5 (*t*, C-3′), 31.9 (*t*, C-11′), 29.5, 29 3, (*t*, C-5 to C-10′), 25.3 (*t*, C-4′), 22.6 (*t*, C-12′), 21.0 (*q*, MeCOO), 14.0 (*q*, C-13′). UV $\lambda_{max}^{MeOH}$ nm (log ε). 300 (3.7), 260 (4.0), 220 (4.2). $[\alpha]_D^{22}$ −15.2° (MeOH, *c* 0 99). EIMS 70 eV, *m/z* (rel. int.): 422 [M]$^+$ (12), 363 (23), 362 (88), 330 (14), 207 (34), 206 (40), 204 (12), 203 (16), 196 (71), 195 (15), 193 (16), 191 (26), 190 (72), 189 (13), 178 (13), 177 (15), 165 (27), 164 (100).

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