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N-ARACHIDYLANTHRANILIC ACID, A NEW DERIVATIVE FROM *ONONIS NATRIX*

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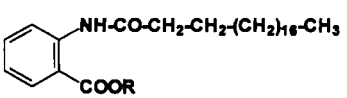
ABSTRACT.—From the $CHCl_3$ extract of the aerial parts of *Ononis natrix*, a new natural product, *N*-arachidylanthranilic acid [**1**] has been isolated, in addition to the known compounds, gardenin B, xanthomicrol, hymenoxin, 8-hydroxy-6-methoxy-3-undecyl-3,4-dihydroisocoumarin, and medicarpin-β-D-glucoside. The structure of **1** was established by spectroscopic and chemical methods.

The genus *Ononis* belongs to the family Leguminosae (tribe Trifoleae) and comprises more than 75 annual or perennial species, indigenous to Eurasia, especially the Mediterranean region (1,2). *Ononis natrix* L. is a perennial herb distributed throughout Jordan (1,3). Infusions of the roots and flowers of *O. natrix* have been used for the treatment of certain disturbances of the urinary tract and have been reported to have diuretic and antirheumatic properties (4). Compounds isolated from the genus *Ononis* have also shown antibiotic and molluscicidal activities (5). The genus *Ononis* is known to produce triterpenoids (2), anthranilic acid derivatives (5), resorcinol derivatives (4–6), dihydroisocoumarins (4,7), a homopterocarpin (7), aromatic lactones (8), flavonoids, and isoflavonoids (5,6). In this paper we describe the isolation and determination of the structure of a new product, namely, *N*-arachidylanthranilic acid [**1**].

From the $CHCl_3$ extract of the aerial parts of *Ononis natrix*, five known compounds were isolated, namely, gardenin B, xanthomicrol, hymenoxin, 8-hydroxy-6-methoxy-3-undecyl-3,4-dihydroisocoumarin, and medicarpin-β-D-glucoside, which were identified by direct comparison with authentic samples and/or with reported physical and spectral data (4, 9–14). In addition, the novel *N*-arachidylanthranilic acid [**1**] was isolated. This is the first report of the isolation of gardenin B, xanthomicrol, hymenoxin, and medicarpin-β-D-glucose from this genus. Xanthomicrol has shown antispasmodic activity by inhibition of the jejunum in test animals (11), and antimicrobial activity against *Aspergillus parasiticus, Candida tropicalis,* and *Fusarium solani* (15). Hymenoxin was found to be cytotoxic to cultured human cells (13). Medicarpin-β-D-glucoside has been previously isolated from the roots of alfalfa (*Medicago sativa* L.) (14,16), but the ^{13}C-nmr data of this substance are reported here for the first time.



1 R=H
2 R=CH_3

The structure of **1** was assigned through interpretation of its spectroscopic properties. The ^1H-nmr spectrum showed signals due to a system of four vicinal aromatic protons at δ 8.77 (dd, $J_{3,4}$=8 Hz, $J_{3,5}$=2 Hz, H-3), 8.13 (dd, $J_{6,5}$=8 Hz, $J_{6,4}$=2 Hz, H-6), 7.60 (ddd, $J_{4,3}$=8 Hz, $J_{4,5}$=7 Hz, $J_{4,6}$=2 Hz, H-4), 7.11 (ddd, $J_{5,6}$=8 Hz, $J_{5,4}$=7 Hz, $J_{5,3}$=2 Hz, H-5), together with 32 aliphatic protons

appearing between δ 1.24–1.42. In addition, two protons appearing as a triplet at δ 2.47 were assigned to those α- to the amide; the two protons which appeared as a quartet at δ 1.77 were assigned to those positioned β- to the amide. The triplet at δ 0.88 (J=7 Hz) was assigned to the terminal methyl group, and a proton of a secondary amide, which appeared at low field as a broad singlet, was observed at δ 10.93 (5,17). The couplings in the ^{1}H-nmr spectrum of **1** were confirmed by COSY nmr experiments. The methylated derivative **2** showed a similar ^{1}H-nmr spectrum to that of **1**, with the only difference being the singlet signal at δ 3.90 due to the methyl ester of an aromatic acid group. A molecular formula of $C_{27}H_{45}O_3N$ deduced from the ms for **1** was suggested by the molecular ion at *m/z* 431, and the number of carbons was confirmed by the ^{13}C-nmr spectrum with the aid of HETCOR nmr experiments. The base peak of compound **1** appeared at *m/z* 137, corresponding to free anthranilic acid. This can be interpreted as a result of a McLafferty transposition of an aromatic amide after the loss of an alkylketene from the fragment ion at *m/z* 179 (5,17), and the base peak of the methyl derivative **2** appeared at *m/z* 151, corresponding to methyl anthranilate. The ir spectrum of **1** showed strong absorptions of the ortho disubstituted benzene ring (1608, 1590, 1455, 755 cm^{-1}), the amide group (3350, 1676, 1535 cm^{-1}), and the aromatic acid (1705, 1418 cm^{-1}) (5,17). Its uv spectrum showed strong absorptions at 253 and 303 nm, consistent with compound **1** being an anthranilic acid derivative (5). With these data and through comparison with those reported for *N*-Δ^{13}-docosenoylanthranilic acid (5) and for *N*-docosenoylanthranilic acid (17), we propose the structure of *N*-arachidylanthranilic acid (*N*-eicosenoylanthranilic acid) for compound **1**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Stuart melting-point apparatus and are uncorrected. Ir spectra were determined with KBr pellets on a Jasco IR-810 spectrophotometer. Uv spectra were determined on a Unicon 810 spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were measured on a JEOL GX-270 spectrometer using TMS as internal standard; chemical shifts are reported in δ (ppm) units. Low-resolution ms were recorded on a Varian MAT model CH-5 spectrometer. Si gel was used for cc (Kieselgel 60, Merck) and tlc (Kieselgel $60F_{254}$).

PLANT MATERIAL.—The aerial parts of *Ononis natrix* were collected in the vicinity of Dahal, 45 km north of Amman, Jordan, in June 1991. A voucher specimen has been deposited at the Herbarium of the Department of Biological Sciences, Faculty of Science, University of Jordan, Amman, Jordan.

EXTRACTION AND ISOLATION.—Powdered, air-dried aerial parts of *Ononis natrix* (17 kg) were repeatedly extracted (4×) by percolation with EtOH for 10 days (each 20 liters). After the solvent was evaporated, a syrupy residue (916.4 g) was obtained, suspended in H_2O (2 liters), and extracted successively with Et_2O (1 liter ×3) (fraction A, 180 g), $CHCl_3$) (1 liter×3) (fraction B, 350 g), and *n*-BuOH (1 liter×3) (fraction C, 55 g). Fraction B (350 g) was dissolved in petroleum ether/$CHCl_3$ (2%) (100 ml) and chromatographed over a Si gel (700 g) column (column A) eluted with varying proportions of petroleum ether, $CHCl_3$, and MeOH mixtures to afford fractions which were collected (100 ml) and combined according to tlc analysis (Me_2CO-$CHCl_3$-MeOH, 5:3:2).

Gardenin B.—Elution of the column (column A) with petroleum ether-$CHCl_3$ (8:2 to 6:4) afforded a solid residue (50 g), which showed two major spots on tlc. The residue was rechromatographed over a Si gel (150 g) column (column B) in petroleum ether-EtOAc (8:2), and the polarity was gradually increased by the addition of EtOAc and EtOAc/MeOH. Gradient elution of column B with petroleum ether-EtOAc (4:6 to 2:8) and EtOAc afforded a solid residue (450 mg) that was recrystallized from MeOH to give gardenin B (300 mg), mp 151°. This compound was identified as 5-hydroxy-6,7,8,4′-tetramethoxyflavone by direct comparison (uv, ir, ^{1}H nmr, ms) with literature data (9,10).

Xanthomicrol.—Continued elution of column B with EtOAc-MeOH (97:3 to 9:1) afforded a residue (4.3 g) which was dissolved in hot MeOH to furnish xanthomicrol (2 g), mp 228°. This isolate was identified as 5,4′-dihydroxy-6,7,8-trimethoxyflavone on the basis of spectral and physical data comparison (mp, mmp, uv, ir, ^{1}H nmr, ms, co-tlc) with an authentic sample (11), and with reported values in the literature (12).

8-Hydroxy-6-methoxy-3-undecyl-3,4-dihydro-

isocoumarin.—Continued elution of column A with petroleum ether-$CHCl_3$ (2:8) and $CHCl_3$ furnished a residue (45 mg) that, upon crystallization from MeOH, yielded 8-hydroxy-6-methoxy-3-undecyl-3,4-dihydroisocoumarin (28 mg), mp 97°, a known constituent of *O. natrix*, identified by direct comparison with previously reported spectral data (uv, ir, ^{1}H nmr, ms) (4).

Hymenoxin.—Continued elution of column A with $CHCl_3$-MeOH (97:3 to 94:6) afforded a solid residue (1.3 g) which, upon treatment with MeOH, yielded hymenoxin (1 g), mp 223–224°. This compound was identified as 5,7-dihydroxy-6,8,3′,4′-tetramethoxyflavone on the basis of spectral data comparison (uv, ir, ^{1}H nmr, ms) with reported values (12,13).

Medicarpin-β-D-glucoside.—Continued elution of column A with $CHCl_3$-MeOH (9l:9 to 85:15) afforded a solid residue (18.3 g), which showed two major spots on tlc. This residue was rechromatographed over Si gel (75 g) (column C) in EtOAc-MeOH (97:3). Elution of the column with EtOAc-MeOH (94:6 to 91:9) afforded a solid residue (56.2 mg) which, upon treatment with MeOH, yielded medicarpin-β-D-glucoside (38 mg), mp 272–273°; ^{13}C nmr ($CDCl_3$, 100 MHz) δ 65.9 (C-2), 40.1 (C-3), 77.7 (C-4), 131.9 (C-5), 110.3 (C-6), 158.4 (C-7), 104.0 (C-8), 156.2 (C-9), 114.1 (C-10), 119.2 (C-1′), 125.1 (C-2′), 106.0 (C-3′), 160.5 (C-4′), 96.3 (C-5′), 160.2 (C-6′), 100.3 (C-1″), 73.1 (C-2″), 77.0 (C-3″ or C-5″), 69.6 (C-4″), 76.5 (C-5″ or C-3″), 60.6 (C-6″), 55.2 (OMe). This isolate was identified as (−)-3-β-D-glucosyl-9-methoxypterocarpan, a known constituent of *Medicago sativa* roots, by direct comparison with previously reported spectral data (uv, ir, [α]D, ^{1}H nmr, ms) (14).

N-*Arachidylanthranilic acid* [**1**].—Continued elution of column C with EtOAc-MeOH (85:15 to 8:2) afforded a solid residue which, on crystallization from MeOH, yielded *N*-arachidylanthranilic acid [**1**] (42 mg), mp 86–87°; uv (MeOH) λ max (log ε) 253 (4.9), 303 (4.6) nm; ir ν max 3350, 1705, 1676, 1608, 1590, 1535, 1475, 1455, 1418, 1282, 1182, 920, 780, 755 cm^{-1}; eims *m/z* 431 (2) (measured 431.3399, calcd 431.3390 for $C_{27}H_{45}O_3N$), 413 (7), 179 (29), 161 (72), 137 (100), 119 (20); ^{1}H nmr ($CDCl_3$) δ 0.88 (3H, t, *J*=7 Hz, H-20′), 1.24–1.42 (32H, m, H-4′–H-19′), 1.77 (2H, q, *J*=7 Hz, H-3′), 2.47 (2H, t, *J*=7 Hz, H-2′), 7.11 (1H, ddd, *J*=8, 7, and 2 Hz, H-5), 7.60 (1H, ddd, *J*=8, 7, and 2 Hz, H-4), 8.13 (1H, dd, *J*=7 and 2 Hz, H-6), 8.77 (1H, dd, *J*=8 and 2 Hz, H-3), 10.93 (1H, br s, -NH-); ^{13}C nmr ($CDCl_3$, 100 MHz) δ 114.5 (C-1), 142.4 (C-2), 121.0 (C-3), 135.9 (C-4), 123.0 (C-5), 132.0 (C-6), 172.4 (C-1′ or COOH), 39.0 (C-2′), 23.0 (C-3′), 25.9–32.3 (C-4′ to C-19′), 14.4 (C-20′), 173.2 (COOH or C-1′).

Methylation of **1**.—A sample of **1** (8 mg) was treated with CH_3I/K_2CO_3 in Me_2CO under reflux for 3 h. The mixture was suspended in H_2O and extracted with EtOAc, the EtOAc solution was evaporated, and the residue was subjected to prep. tlc (*n*-hexane-EtOAc-MeOH, 8:2:1), to afford compound **2** (5 mg) as an oily liquid. Eims *m/z* 445 (11), 417 (25), 389 (58), 358 (33), 193 (25), 151 (100), 119 (15); ^{1}H nmr ($CDCl_3$) δ 0.88 (3H, t, *J*=7.2 Hz, H-20′), 1.25–1.43 (32H, m, H-4′–H-19′), 1.77 (2H, q, *J*=7.2 Hz, H-3′), 2.46 (2H, t, *J*=7.2 Hz, H-2′), 3.90 (3H, s, OMe), 7.08 (1H, ddd, *J*=8, 7, and 2 Hz, H-5), 7.55 (1H, ddd, *J*=8, 7, and 2 Hz, H-4), 8.04 (1H, dd, *J*=7 and 2 Hz, H-6), 8.74 (1H, dd, *J*=8 and 2 Hz, H-3), 11.05 (1H, br s, -NH-).

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