Journal of Natural Products
Vol. 53, No. 1, pp. 167-170, Jan-Feb 1990

MONOGLYCOSIDES FROM *ISOPLEXIS CHALCANTHA*

JUAN M. TRUJILLO,* OLGA HERNANDEZ,

Instituto de Productos Naturales Orgánicos, C.S.I.C., La Laguna, Tenerife, Canary Islands, Spain

and EDUARDO NAVARRO

Departamento de Farmacología, Facultad de Medicina, Universidad de La Laguna, Tenerife, Canary Islands, Spain

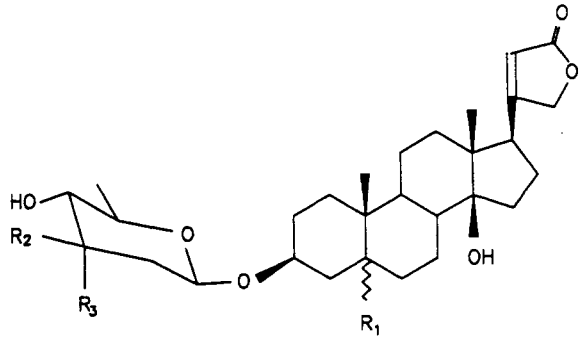
ABSTRACT.—Three monoglycosides, uzarigenin-3β-*O*-canaroside [**1**], digitoxigenin-3β-*O*-digitoxoside [**2**], and uzarigenin-3β-*O*-digitoxoside [**3**], were isolated from the C_6H_6 extract of leaves of *Isoplexis chalcantha*. Their ir, ^{1}H-nmr, ^{13}C-nmr, and ms spectroscopic data are presented. Their identity was also established by comparison with the products of enzymatic hydrolysis of diglycosides present in the plant.

A C_6H_6 extract obtained from the leaves of *Isoplexis chalcantha* Svent. & O'Shan. (Scrophulariaceae) (1), collected in the gardens of the Instituto de Productos Naturales Orgánicos of La Laguna in 1984, was fractionated by cc. After separation of the genins, monoglycosides, and diglycosides in the extract, the cardenolide monoglycosides were further purified and identified. In order of decreasing polarity, these were uzarigenin-3β-*O*-canaroside [**1**], digitoxigenin-3β-*O*-digitoxoside [**2**], and uzarigenin-3β-*O*-digitoxoside [**3**]. The structures of these monoglycosides were elucidated by spectroscopic techniques and by acid hydrolysis of the compound to products identified by chromatography (pc, tlc, and glc) and comparison with authentic samples. The enzymatic hydrolysis of diglycosides present in the C_6H_6 extract also led to the corresponding monoglycosides (pc, tlc).

Uzarigenin-3β-O-*canaroside* [**1**].—This compound crystallized from MeOH and gave a positive Kedde's reaction (2), showing the presence of an α,β-unsaturated γ-lactone. Its ir spectrum displays a strong absorption at 3400 cm^{-1}, corresponding to hydroxyl groups, and another band at 1738 cm^{-1} typical of the absorption of the carbonyl group of the α,β-unsaturated γ-lactone ring.

The ms spectrum had a molecular ion at *m/z* 504, and also an ion at *m/z* 374. This latter ion corresponds to the free genin, as is frequently observed with this type of compound (3).

In the ^{13}C-nmr spectrum a signal at δ 17.91 was observed, corresponding to a methyl group. This downfield position



1 R_1=αH, R_2=OH, R_3=H
2 R_1=βH, R_2=H, R_3=OH
3 R_1=αH, R_2=H, R_3=OH

is typical of methyls of 6-deoxy sugars. A signal at δ 97.48 is typical of the anomeric carbon of the sugar, and three other signals at δ 71.77, 72.13, and 77.28 were assigned to carbons attached to hydroxy groups. The signal resonating at δ 39.77 was assigned to a methylene group. The compound was thus identified as a 2,6-dideoxy sugar.

The ^{1}H-nmr spectrum displayed three-proton singlets at δ 0.77 and 0.85, located in positions corresponding to those given for uzarigenin. A doublet (J = 6 Hz) appeared at δ 1.31, integrating for three protons, and was attributable to the C-6′ methyl group of the sugar. Two protons corresponding to H-3′ and H-4′ were located between δ 3.13 and 3.23, whereas H-5′ was centered at δ 3.99 (t, J = 5.7 Hz). The remainder of the signals had similar shapes and positions to those reported for uzarigenin.

Acid hydrolysis of a small amount of this compound afforded a substance which was identical with uzarigenin by comparison (tlc) with an authentic sample, in addition to a sugar identified as canarose by gc (4). Enzymatic hydrolysis of the diglycoside uzarigenin-3β-*O*-glucosylcanaroside (5) gave, in addition to glucose, a monoglycoside, the physical and spectroscopic data of which coincide totally with the monoglycoside **1** isolated by us. These data confirm the structure uzarigenin-3β-*O*-canaroside [**1**] for the first glycoside; this is the first report of this compound in nature.

Digitoxigenin-3β-O-*digitoxoside* [**2**].—This compound was crystallized from MeOH. Its ir spectrum displayed the signals characteristic of hydroxyl groups at 3600 cm^{-1} and of the α,β-unsaturated lactone at 1740 cm^{-1}. Its ms exhibited the highest peak at *m/z* 401 and an ion for the genin was observed at *m/z* 374.

The ^{13}C-nmr spectrum (6) showed the methyl of the sugar at δ 18.31. The signals corresponding to C-3′, C-4′, and C-5′ appeared at δ 68.47, 72.88, and 69.4, while the signal of C-1′ resonated at δ 95.6. The remainder of the signals were at the position given in the literature for digitoxigenin (7). The ^{1}H-nmr spectrum showed two three-proton singlets at δ 0.83 and 0.90, corresponding to the C-18 and C-19 angular methyls, and a doublet at δ 1.26 assignable to the methyl of the sugar. The remainder of the signals corresponding to the glycoside protons appeared between δ 3.30 and 4.11, the signal of the anomeric proton being included in the signal corresponding to the protons of C-21 of the lactone moiety. The remainder of the signals exhibited the same multiplicity and were located at the same position as those of digitoxigenin, previously isolated from this plant.

The acid hydrolysis of this compound afforded a genin that proved to be identical with digitoxigenin, in addition to the sugar digitoxose, identified by glc. These data identify the glycoside as digitoxigenin-3β-*O*-digitoxoside [**2**]; this compound has previously been isolated by Tschesche *et al.* (8).

Uzarigenin-3β-O-*digitoxoside* [**3**].—This compound was crystallized from MeOH. Its ir spectrum displayed two bands corresponding to hydroxyl groups at 3600 and 3450 cm^{-1} and a band at 1740 cm^{-1} corresponding to the α,β-unsaturated lactone.

The ^{1}H-nmr spectrum, with respect to the protons of the steroidal nucleus, was superimposable on that of uzarigenin-canaroside, previously described. In contrast, the sugar protons were identical with those of digitoxigenin-3β-*O*-digitoxoside [**2**]. Its ^{13}C-nmr spectrum also displayed a great similarity, with regard to the steroidal part, to that of the monoglycoside **1** and, with respect to the glycoside moiety, to the monoglycoside **2**. Its ms showed the highest mass ion at *m/z* 401 and an ion for the genin at 374.

Acid hydrolysis permitted identification, as in all the preceding cases, of uzarigenin and digitoxose. The enzy-

matic hydrolysis of uzarigenin-3β-*O*-glucosyldigitoxoside, also present in the plant, afforded the corresponding monoglycoside, identical with **3**, in addition to glucose. Glycoside **3** has not previously been isolated from nature.

TABLE 1. ^{13}C-nmr spectral data of compounds **1**, **2**, and **3** (50 MHz, $CDCl_3$ in ppm).

Carbon	1	2	3
C-1 (t)	37.4	30.1	37.4
C-2 (t)	29.4	26.8	29.5
C-3 (d)	73.7	73.2	73.3
C-4 (t)	34.4	30.4	34.5
C-5 (d)	44.5	35.5	44.5
C-6 (t)	28.8	27.1	28.8
C-7 (t)	27.0	21.2	27.1
C-8 (d)	41.9	42.1	41.9
C-9 (d)	49.7	35.9	50.1
C-10 (s)	36.1	35.3	36.1
C-11 (t)	21.3	21.6	21.4
C-12 (t)	40.1	40.2	40.1
C-13 (s)	50.1	49.8	49.8
C-14 (s)	85.7	85.8	85.7
C-15 (t)	33.2	33.3	33.3
C-16 (t)	27.6	29.9	27.6
C-17 (d)	51.1	51.1	51.1
C-18 (q)	15.9	15.9	15.9
C-19 (q)	12.3	23.7	12.3
C-20 (s)	174.8	174.9	174.7
C-21 (t)	73.7	73.7	76.3
C-22 (d)	117.8	117.8	117.9
C-23 (s)	174.8	174.8	174.7
C-1′ (d)	97.5	95.6	95.6
C-2′ (t)	39.8	38.4	38.6
C-3′ (d)	71.8	68.5	68.5
C-4′ (d)	77.3	72.9	72.2
C-5′ (d)	72.1	69.4	69.5
C-6′ (q)	17.9	18.3	18.3

EXPERIMENTAL

Melting points were taken on a Kofler block and are uncorrected. Ir spectra were obtained with a Perkin-Elmer 681 spectrophotometer in $CHCl_3$ solutions. The ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker WP200SY spectrometer in $CDCl_3$ solutions using TMS as reference. Optical rotations were measured in MeOH on a Perkin-Elmer 141 polarimeter with a 10-cm microcell. Mass spectra were recorded on a VG Micromass ZAB-2F at 70 eV. Tlc was carried out on Schleicher and Schüll TLC-Ready Foils using EtOAc-MeOH-H_2O (80:5:5) as eluent. Cc was carried out on Merck 60H Si gel (0.063 mesh) at a pressure of 3 atmospheres.

The aerial part of *I. chalcantha* was identified by Dr. Marcelino del Arco. A voucher specimen was deposited at the Herbarium of the Department of Botany, University of La Laguna (TFC 23.453).

Air-dried and finely powdered *I. chalcantha* leaves (1013 g) were exhaustively extracted in a Soxhlet apparatus with solvents of increasing polarity: *n*-hexane, C_6H_6, $CHCl_3$, EtOAc, and MeOH.

Isolation of the compounds.—The C_6H_6 extract (26.9 g) was submitted to cc, using mixtures of *n*-hexane/EtOAc of increasing polarity. The less polar fractions afforded two genins: uzarigenin and digitoxigenin. Compounds **1–3** were isolated from the intermediate fractions. The more polar fractions of this extract yielded three diglycosides previously reported by us (5). The monoglycosides were identified in order of decreasing polarity as uzarigenin-3β-*O*-canaroside [**1**], digitoxigenin-3β-*O*-digitoxoside [**2**], and uzarigenin-3β-*O*-digitoxoside [**3**].

Uzarigenin-3β-O-*canaroside* [**1**].—Colorless needles from MeOH (230 mg), mp 230–235°; $[\alpha]^{25}D$ −22.7 (MeOH); ir cm^{-1} 3580, 3400, 1738, 1612, 1165, 1060, 1020, 895; eims *m/z* (% rel. int.) $[M]^+$ 504 (0.34), $[M-18]^+$ 486 (0.45), $[M-\text{sugar}]^+$ 374 (3.81), 357 (95), 339 (73), 203 (70.2), 131 (100); ^{1}H nmr (200 MHz, $CDCl_3$), δ (ppm) 0.77 (3H, s), 0.85 (3H, s), 1.31 (3H, d, J = 6 Hz), 2.74 (1H, m), 3.13 (1H, t, J = 8.8 Hz), 3.23 (1H, m), 3.57 (1H, m), 3.99 (1H, t, J = 5.7 Hz), 4.60 (1H, dd, J = 9.5 and 2.0 Hz), 4.84 (2H, dd, J = 20.0 and 1.6 Hz), 5.85 (1H, t, J = 1.6 Hz).

Digitoxigenin-3β-O-*digitoxoside* [**2**].—Needles from MeOH (180 mg), mp 154–158°; $[\alpha]^{25}D$ −14.5 (MeOH); ir cm^{-1} 3600, 3410, 2930, 1740, 1618, 1070, 1030, 950, 860; eims *m/z* (% rel. int.) 401 (3.3), $[M-\text{sugar}]^+$ 374 (2.8), 357 (100), 339 (93), 246 (35.5), 203 (87.9), 131 (37.8); ^{1}H nmr (200 MHz, $CDCl_3$) δ (ppm) 0.85 (3H, s), 0.90 (3H, s), 1.26 (3H, d, J = 6.0 Hz), 2.77 (1H, m), 3.33 (1H, dd, J = 9.0 and 3.0 Hz), 3.71 (1H, m), 4.01 (1H, s), 4.11 (1H, q, J = 3.0 Hz), 4.73–5.03 (3H, m), 5.85 (1H, s).

Uzarigenin-3β-O-*digitoxoside* [**3**].—Needles from MeOH (340 mg), mp 222–226°; $[\alpha]^{25}D$ −17.5° (MeOH); ir cm^{-1} 3600, 3450, 2930, 1740, 1615, 1160, 1130, 1005, 895, 860; eims *m/z* (% rel. int.) 401 (4), $[M-\text{sugar}]^+$ 374 (2.3), 357 (100), 339 (80), 246 (4.8), 231 (8.3), 203 (88.9), 131 (48); ^{1}H nmr (200 MHz, $CDCl_3$) δ (ppm) 0.76 (3H, s), 0.84 (3H, s), 1.27 (3H, d, J = 6 Hz), 2.73 (1H, m), 3.31 (1H, dd, J = 9.0 and 3.0 Hz), 3.66 (2H, m), 4.10 (1H, d, J = 4.0 Hz), 4.81–5.02 (3H, m), 5.85 (1H, s).

Acid hydrolysis of the monoglycosides.—In separate experiments, 50 mg of each of the monoglycosides **1**, **2**, and **3** were dissolved in Me_2CO (5 ml) and distilled H_2O (5 ml), and H_2SO_4 0.5 N (1 ml)

was added. The solution was heated under reflux for 4 h, extracted three times with EtOAc (15 ml), and treated in the usual manner. Tlc analysis of the organic extract permitted identification of uzarigenin in the monoglycosides **1** and **3**, as well as digitoxigenin in **2**.

Enzymatic hydrolysis of the diglycosides.—From the more polar fractions of the C_6H_6 extract, three glycosides were isolated in order of decreasing polarity and identified with uzarigenin-3β-*O*-glucosylcanaroside, uzarigenin-3β-*O*-glucosyldigitoxoside, and digitoxigenin-3β-*O*-glucosyldigitoxoside, all of which have glucose as the terminal sugar. These glycosides were named diglycosides A, B, and C, respectively. Each of these products (20 mg) was separately refluxed in H_2O (120 ml) until totally dissolved and kept until room temperature was attained; β-glucosidase (40 mg) was added and the mixture incubated for 12 days at 37°. The solution was concentrated in vacuo until its volume was reduced by half, EtOH (30 ml) was added, the mixture was boiled for several minutes and centrifuged, and the excess of EtOH was removed in vacuo. The residue was extracted three times with EtOAc; the organic layer was washed three times with H_2O and dried over Na_2SO_4.

The products resulting from this hydrolysis were identified as the monoglycosides **1**, **2**, and **3** by their physical constants and spectroscopic data, in addition to comparison with those obtained from the plant.

LITERATURE CITED

1. E.R. Sventenius, in: "Index Seminum. Hortus Acclimatations Plantarum Auratapae." INIA-MAPA, Madrid, 1968, pp. 43-60.
2. R. Tschesche, G. Crimmer, and F. Seehofer, *Chem. Ber.*, **86**, 1235 (1953).
3. P. Reichstein, W. Stocklin, and T. Reichstein, *Helv. Chim. Acta*, **50**, 2139 (1967).
4. B. Kopp, J. Jurenitsch, and W. Kubelka, *J. Chromatogr.*, **210**, 219 (1981).
5. A.G. González, J. Bretón, E. Navarro, J. Trujillo, J. Boada, and R. Rodríguez, *Planta Med.*, 9 (1985).
6. W. Robien, B. Kopp, D. Schabl, and H. Schwarz, in: "Progress in Nuclear Magnetic Resonance Spectroscopy." Ed. by J.W. Emsley, J. Feeney, and L.H. Sutcliffe, Pergamon Press, 1987, Vol. 19, pp. 131–181.
7. D. Kruger, P. Junior, and M. Wichtl, *Planta Med.*, **49**, 74 (1983).
8. R. Tschesche, H. Wirtz, and G. Snatzke, *Ber.*, **88**, 1619 (1955).

Received 26 April 1989