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NORDITERPENES AND NORDITERPENE GLYCOSIDES FROM *DRYMARIA ARENARIOIDES*

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Key Word Index—*Drymaria arenarioides*, Caryophyllaceae, norditerpenes, norditerpene glycosides

Abstract—From the aerial parts of *Drymaria arenarioides*, two previously known compounds, atractyligenin and 2-*O*-β-D-glucopyranosyl atractyligenin, and two new natural substances, atractyligenin 2-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside and atractyligenone (2-*oxo*-15α-hydroxyatractyl-16,17-ene-4α-carboxylic acid) have been isolated Their structures were established by chemical and spectroscopic techniques as well as comparison with authentic samples.

INTRODUCTION

Continuing our search for biologically active compounds of natural origin, we have undertaken an investigation of *Drymaria arenarioides* Willd. (Caryophyllaceae). Known as 'alfombrilla' in the semidesert areas of northern Mexico and southern United States, the plant is said to be responsible for the death of livestock that accidentally feed on it It has been previously reported that the saponin-containing extract of *D arenarioides* was toxic to sheep and chicks in the laboratory [1] Previous work on the genus has not been very extensive Triterpenoids [2] and mixtures of long chain fatty acids [3] have been reported.

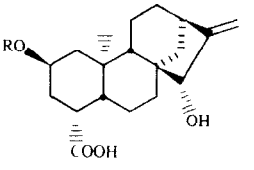
We have found in the course of our research that the methanolic extract of *D. arenarioides* contains norditerpenes **(1,5)** and norditerpene glycosides **(2,3)** of the attractyligenin type [4].

RESULTS AND DISCUSSION

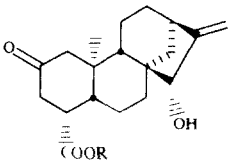
The concentrate from the methanolic extract of the aerial parts of *D. arenarioides* was partitioned between water and *n*-butanol. Successive chromatographic steps applied to the organic portion yielded the known compounds **1** and **2** as well as two others **(3** and **5)** which had not been previously isolated as natural compounds

Compound **1** was identified as atractyligenin, the aglycone of atractyloside, a toxic glycoside first isolated from *Atractylis gummifera* L (Compositae) [5] Its identity was established by comparison of the ^{13}C NMR and mass spectral data with those of the literature [4,6] as well as co-chromatography with an authentic sample. Compound **2**, $C_{25}H_{38}O_9$, was hydrolysed under acidic conditions to give **1** and D-glucose The position of attachment of the sugar was established by comparison of the ^{13}C NMR spectra of **1** and **2** and was assigned as being at C-2. Thus, the identity of **2** was established as 2-*O*-β-D-glucopyranosyl atractyligenin which had been previously reported as one of the 'coffee atractylosides' isolated from *Coffea arabica* beans [7]

The ^{13}C NMR spectrum of compound **3**, $C_{31}H_{48}O_{14}$, presented characteristics similar to that of **2** with additional signals of a second sugar moiety Acid hydrolysis of **3** yielded atractyligenin **(1)** and D-glucose The FABMS (negative ion) of **3** showed a quasimolecular ion at *m/z* 643 $[M-H]^-$ indicating a molecular weight of 644 Additionally, in the D/CIMS the following fragments could be observed *m/z* 662 $[M+NH_4]^+$, 500 $[(M+NH_4)-162]^+$, 320 $[M-(2x162)]^+$, thus confirming the fragmentation of two D-glucose units The nature of the interglycosidic linkage was established by comparison of the ^{13}C NMR spectra of **2** and **3.** The assignments of the sugar carbon atoms are given in Table 1 Evidence



1 R = H
2 R = glc
3 R = glc$^{1\text{-}2}$glc
glc = β-D-glucopyranosyl



4 R = H
5 R = Me

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Table 1. ^{13}C NMR data for compounds 2–4 (50.1 MHz in pyridine-d_5)*

C	2	3	4
1	48.36	48.37	56.55
2	72.63	74 19	208.54
3	36 77	35.66	43 92
4	44 47	44.48	46 07
5	49 81	49.81	48.33
6	26 59	26.58	26 21
7	35.62	36 09	36 61
8	48.48	48 37	48 06
9	53.63	53 64	52 59
10	41.20	41 21	43 50
11	18.68	18.68	18 50
12	33.01	33 01	32 70
13	42.92	42 93	42 74
14	36.10	36 78	36 10
15	82 73	82 75	82 20
16	160 98	161 05	160 71
17	107 81	107.86	107 99
19	177 87	177 90	177.15
20	17 14	17 24	17.68
1′	103 23	102 76	
2′	75 55	88 95	
3′	78.49	78 78	
4′	71.85	71.76†	
5′	78.76	78.33‡	
6′	62 96	62.68§	
1″		106 10	
2″		72.88	
3″		77 95	
4″		69 94†	
5″		75.75‡	
6″		62 54§	

*Carbon multiplicities were determined by the use of DEPT experiments

†,‡,§ Signals may be interchanged

of a C-2'-glycosidic linkage arises from the fact that the C-2' in **3** is shifted downfield by about 13 ppm to δ88.95 relative to that of the C-2' carbon in **2** (δ75.55). This is in agreement with previous observations of the glycosidation pattern of saponins [8,9]. Thus, the proposed structure for **3** is that of atractyligenin 2-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside, to our knowledge a new natural compound

The last diterpenoid isolated from *D. arenarioides* was compound **4**, $C_{19}H_{26}O_4$, which presented a ^{13}C NMR spectrum similar to that of atractyligenin (**1**). The main difference between the two compounds is found in the appearance of a new signal for a C=O carbon at δ208 5 (IR: 1695 cm^{-1}) and the disappearance of the signal corresponding to C-2 carbon atom in atractyligenin at δ63 93. Accordingly, we observed a downfield shift of the corresponding C-1 and C-3 carbons to δ56 1 and 42 8, respectively, which is in agreement with the α-effect expected when changing from an sp^3 C–O to an sp^2 C=O The EIMS of **4** gave an M^+ signal at *m/z* 318, which corresponds to a difference of 2H units with respect to atractyligenin, thus confirming that **4** is the keto derivative of **1**. Comparison of the ^{13}C NMR spectra of **4** and that reported for the synthetic methyl ester (**5**)[6] provides further confirmation that the proposed structure for atractyligenone is **4**

Summarizing, we have isolated from the 'saponin-containing' extract of *D. arenarioides* two norditerpenes and two norditerpene glycosides as part of its main constituents Two of these compounds (**3** and **4**) are, to our knowledge, reported here for the first time as natural products. It is likely that these substances are in part responsible for the toxicity previously reported [1], since we have not yet detected saponins in significant amounts from the methanol extract, although it was previously described as being saponin-rich [1] In addition, this is also the first report on the polar constituents of the genus *Drymaria*.

EXPERIMENTAL

General. Mps uncorr CC employed silica gel 60 (63–200 μm, Merck) Centrifugal TLC was performed on a Chromatotron instrument. Low pressure LC was done on a Lobar LiChroprep Si 60 column (40–63 μm, 27 × 2 5 cm, Merck) Centrifugal partition chromatography (CPC) was carried out on a CPC model LLN (Sanki Engineering Ltd) apparatus D/CIMS was done on a Ribermag-R10-1013 quadrupole instrument with NH_3 as reactant gas. FABMS were measured on a ZAB 1S spectrometer using a thioglycerol matrix and bombarded with 5 keV Xe atoms. EIMS were measured at 70 eV ^{13}C NMR spectra were recorded on a Varian VXR-200 instrument at 50.1 MHz using pyridine-d_5 as solvent

Plant Material Drymaria arenarioides Willd (Caryophyllaceae) was collected in October, 1985 in Delicias, Chihuahua, Mexico Voucher specimens have been deposited at the Instituto Tecnologico y de Estudios Superiores de Monterrey

Extraction and isolation The air-dried aerial plant material (3 57 kg) was Soxhlet extracted with MeOH, providing 125 g of a greenish residue A portion of the crude syrup (10 g) was partitioned between *n*-BuOH and H_2O. The organic soln was concd under vacuum and lyophilized, providing 5 2 g of a powder-like residue Flash CC of the *n*-BuOH portion (5 g) with $CHCl_3$–MeOH mixtures of increasing polarity provided 28 fractions of 200 ml each

Fractions 5 and 6 (95 mg) were subjected to centrifugal TLC with $CHCl_3$–MeOH (17 3) yielding 45 mg of atractyligenin (**1**). Fractions 10–12 (230 mg) were further separated by CPC using a $CHCl_3$–MeOH H_2O (5 6·4) system in the descending mode giving 26 mg of **2** and 28 mg of **4**. Fractions 16–19 (180 mg) were submitted to low pressure chromatography on silica gel using $CHCl_3$–MeOH–H_2O (58 35 7) giving 18 mg of **2** and 22 mg of **3**. All compounds underwent a final purification step on Sephadex LH-20 Acid hydrolysis of the glycosides was done according to ref [10]

Atractyligenin 2-O-β-D-*glucopyranosyl*-(1→2)-β-D-*glucopyranoside* (**3**). $C_{31}H_{48}O_{14}$, white powder, M_r = 644, mp 249–252° FABMS (negative ion mode, thioglycerol) *m/z* 643 $[M-H]^-$, 481 $[(M-H)-162]^-$. D/CIMS (NH_3, positive ion) *m/z* 662 $[M+NH_4]^+$, 500 $[(M+NH_4)-162]^+$, 320 $[M-(2\times162)]^+$.

Atractyligenone (2-oxo-15α-*hydroxyatractyl*-16,17-*ene*-4α-*carboxylic acid*) (**4**). $C_{19}H_{26}O_4$, white powder, M_r = 318, mp 175–177°, IR ν_{max}^{KBr} (cm^{-1}) 3300–2400, 1720 (COOH), 1695 (C=O) EIMS *m/z* (rel int):318 (100) $[M]^+$, 303 (14), 275 (12), 260 (84), 245 (32), 216 (13), 202 (11), 167 (16), 144 (19), 123 (13), 107 (48), 91 (75), 67 (20), 55 (25)

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A NEW TAXANE DERIVATIVE FROM THE HEARTWOOD OF *TAXUS MAIREI*

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Key Word Index—*Taxus mairei*, Taxaceae, diterpene

Abstract—A new taxane derivative, 9α,10β, 13α-triacetoxy-5α-cinnamoyoxytaxa-4(20),11-diene, has been isolated from the heartwood of *Taxus mairei*. Its structure has been elucidated on the basis of spectroscopic studies.

INTRODUCTION

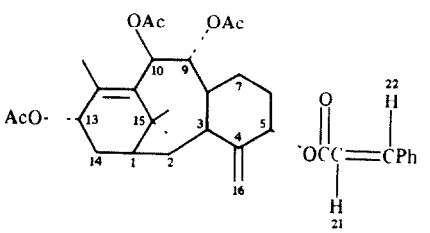
The Taiwan yew, *Taxus mairei*, which is the only species belonging to *Taxus* Linn found in Taiwan, grows wild in the remote mountains up to an elevation of 2000–2300 m[1]. In our reinvestigation[2] of the heartwood of this plant a taxane derivative (**1**) has been isolated. Its structure was elucidated on the basis of infrared, ^{1}H NMR, ^{13}C NMR and mass spectrometry to be the 9,10,13-triacetate, 5α-cinnamate of taxa-4(20),11-dien-5α,9α,10β,13α-tetrol.

RESULTS AND DISCUSSION

The methanolic extract of the heartwood of *Taxus mairei* was extracted several times with *n*-hexane. Upon chromatography, a new taxane derivative **1** was isolated from the *n*-hexane soluble fraction.

The IR spectrum of **1** showed typical bands at ν_{max}^{KBr} 1735 (C=O), 1710 (C=O), 1634 (C=C) cm^{-1}. Its mass spectrum showed a parent ion [M$^+$] at *m/z* 592, the accompanying peak at *m/z* 444 was related to the loss of a cinnamic acid moiety ($C_9H_8O_2$) from the parent ion. The prominent fragments at *m/z* 384 [444–60], 324 [384–60]$^+$ and 284 [324–60]$^+$ indicated that **1** contained three acetate groups.

The 400 MHz ^{1}H NMR spectrum of **1** in $CDCl_3$ showed three methyl singlets at δ2.04, 2.00 and 1.72. These peaks were all assigned to acetyl methyl. Two one-proton doublets at δ7.76 and 6.58 were attributed to H-22 and H-21 ($J_{21,22}$ = 16.0 Hz) [3]. Additionally, a three-proton multiplet at δ7.40 and a two-proton multiplet at 7.49 were assigned to the phenyl protons of the cinna-



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