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A DAMMARANE FROM *STEVIA SALICIFOLIA**

RACHEL MATA,† VERÓNICA RODRÍGUEZ, ROGELIO PEREDA-MIRANDA, ROBERT BYE‡ and EDELMIRA LINARES‡

Laboratorio de Fitoquímica, Departamento de Farmacia, Facultad de Química, Universidad Nacional Autóñoma de México, Coyoacán 04510, México, D.F., México; ‡Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México, Coyoacán 04510, México, D.F., México

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Key Word Index—*Stevia salicifolia*; Asteraceae; roots; triterpenoid; (20*S*)-dammar-13(17),24-diene-3β-yl acetate.

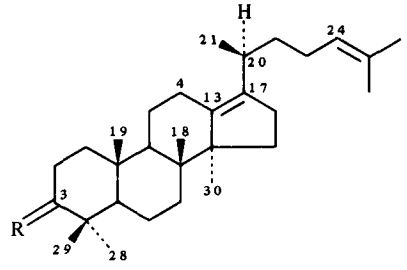
Abstract—A new dammarane was isolated from the hexane extract of the roots of *Stevia salicifolia*. The new metabolite was characterized by spectroscopic and chemical methods as (20*S*)-dammar-13(17),24-diene-3β-yl acetate.

INTRODUCTION

Continuing our research on Mexican medicinal plants we have investigated the roots of *Stevia salicifolia* Cav. (Tarahumara names: 'ronino' and 'roninowa'). An infusion of the roots is drunk by the Tarahumara Indians to alleviate gastrointestinal upsets caused by parasitic deseases, whereas the decoction is used as a cathartic [1]. Previous chemical work on the plant resulted in the isolation and characterization of several aromatic and terpenoid type compounds [2–4].

RESULTS AND DISCUSSION

Column chromatography on silica gel of the hexane extract of dried roots of *S. salicifolia* allowed the isolation of a new naturally occurring dammarane (**1**). Compound **1**, $C_{32}H_{52}O_2$, mp 56–59°, was obtained as needles and responded positively to tetranitromethane test for triterpenoids. Alkaline hydrolysis of **1** afforded derivative **2**, which was treated with Jones reagent to yield the ketone **3**, thus indicating the presence of an acetoxyl functionality in **1**. The mass spectrum of **2** exhibited important ions associated with the dammarane series [5]. Cleavage of ring C gave the diagnostically important peak at *m/z* 207, which excluded the possibilities of either an euphane or a lanostane type of skeleton for **1**. The ^{13}C NMR data of **1–3** (Table 1) supported the assignment of a dammarane-type triterpenoid [6, 7]; the ^1H NMR spectrum of **1** showed signals for one vinylic proton (δ 5.09, *t*, $J=7.3$ Hz), one acetyl moiety (δ 2.06), five tertiary (δ 0.75, 0.88, 0.90 and 0.98), one secondary (δ 0.85, *d*, $J=6$ Hz) and two vinylic (δ 1.60 and 1.68) methyl groups; in addition, a multiplet (*dd*, $J=10$, 6 Hz) assignable to an axial methine proton geminal to an acetoxy functionality in



	R
1	αH,βOAc
2	αH,βOH
3	O

C-3 was observed at δ 4.50 [8]. As expected, this resonance was diamagnetically shifted at δ 3.25 in derivative **2**. Further support for the C-3 substitution was obtained from the ^{13}C NMR data analysis of **3** (Table 1); the observed shifts for C-28 (δ 26.67, $\Delta\delta=-1.3$) and C-29 (δ 21.10, $\Delta\delta=5.0$), caused by the presence of a vicinal ketone group, were consistent with those published for related triterpenes [6, 7].

The CD curve of **3** showed a positive Cotton effect in agreement with the dammarane skeleton [9]. The ORD positive plain curve of **2**, calculated using the olefin octant rule [10] supported the (*S*)-stereochemistry at the C-20 chiral centre. Consistent with this proposal were the ORD values observed for related triterpene alcohols [11]. Therefore, the structure of the natural product **1** is (20*S*)-dammar-13(17), 24-diene-3β-yl acetate.

EXPERIMENTAL

Plant material. The roots of *S. salicifolia* Cav. were collected by R. Bye in Municipio Guachochic, Cusarare, Estado de Chihuahua, México, in November 1988. Voucher specimens (R. Bye No 16657) are deposited at the ethnobotanical collection of the National Herbarium, Instituto de Biología, UNAM.

Extraction and isolation procedures. Dried and finely powdered roots of the plant (2.7 kg) were exhaustively macerated with hexane at room temp. during 4 days. After filtration, the extract

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†Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR chemical shifts of compounds 1–3* ($CDCl_3$, 75.4 MHz)

C	1	2	3
1	37.80	38.89	37.11
2	24.20	27.61	34.57
3	80.89	78.84	218.23
4	37.80	38.87	47.26
5	51.00	50.89	51.45
6	18.77	18.01	20.24
7	34.88	35.19	35.53
8	44.05	44.05	44.07
9	49.59	49.55	49.65
10	37.08	37.20	37.11
11	21.50	21.50	21.41
12	27.48	27.81	27.46
13	133.81	133.95	132.05
14	49.97	49.98	50.09
15	30.81	30.83	30.79
16	28.12	28.13	28.13
17	133.59	133.48	134.07
18	20.18	20.11	19.76
19	15.49	15.50	15.70
20	35.84	35.85	35.85
21	24.42	24.44	24.28
22	35.37	35.35	35.36
23	24.72	24.70	24.75
24	125.16	125.16	125.09
25	130.81	130.86	130.86
26	25.72	25.73	25.75
27	17.67	17.67	17.69
28	27.95	28.01	26.67
29	16.58	15.57	21.10
30	18.89	18.89	18.89
31	21.30	—	—
32	170.99	—	—

*Multiplicities were confirmed by APT spectrum.

was evapd to dryness yielding a residue (215.4 g). Part of the crude extract (200 g) was then subjected to CC on silica gel (1 kg), using hexane with increasing amounts of EtOAc as eluents. Frs. of 500 ml were collected. From frs 14–41, eluted with hexane, crystallized 867.4 mg (0.032% of the dry wt) of **1**, mp 56–58°; $[\alpha]_D$ +32° ($CHCl_3$; *c* 0.015); IR ν^{max} cm^{-1}: 3015, 2950, 2925, 1740, 1465, 1455, 1370, 1250, 1025; ^1H NMR (300 MHz, δ, $CDCl_3$): 0.75 (3H, *s*, H-30), 0.85 (3H, *d*, *J* = 6 Hz, H-21), 0.88 (6H, *s*, H-18, H-28), 0.90 (3H, *s*, H-19), 0.98 (3H, *s*, H-29), 1.60, 1.68 (each 3H, *s*, H-26 and H-27, respectively), 2.06 (3H, *s*, CH_3–CO–), 4.50 (1H, *dd*, *J* = 10, 6Hz, H-3), 5.09 (1H, *t*, *J* = 7.3 Hz, H-24); EIMS (rel. int.): 468 $[M]^+$ (9.3), 453 $[M-15]^+$ (25), 408 $[M-60]^+$ (2), 393 $[M-15-60]^+$ (22), 390 (4), 383 (1), 249 (1), 243 (2), 241 (9), 227 (4), 119 (15), 111 (10), 109 (25), 83 (30), 69 (76.3), 55 (40), 41 (50), 43 (100).

Hyrolysis of compound **1**. Compound **1** (80 mg) was dissolved in 5 ml EtOH and 5 ml 2 M NaOH. The reaction mixt. was refluxed for 6 hr. After usual work-up, the product was recrystallized from EtOH to afford 40 mg of **2**, mp 117–118°; ORD ($CHCl_3$; *c* 0.01) $[\Phi]_{700}$ +37.4, $[\Phi]_{600}$ + 43.8, $[\Phi]_{500}$ +105.6, $[\Phi]_{400}$ +160.1 $[\Phi]_{350}$ +176.3; IR ν_{max} cm^{-1}: 3350, 2950, 1460, 1455, 1380, 1200; ^1H NMR (300 MHz, $CDCl_3$): δ0.75 (3H, *s*, H-30), 0.80 (3H, *s*, H-19), 0.85 (3H, *d*, *J* = 6 Hz, H-21), 0.87 (3H, *s*, H-18), 0.95 (3H, *s*, H-29), 1.00 (3H, *s*, H-28), 1.60, 1.68 (each 3H, *s*, H-26 and H-27, respectively), 3.25 (1H, *dd*, *J* = 10, 6 Hz, H-3), 5.09 (1H, *t*, *J* = 7.3 Hz, H-24); EI *m/z* (rel. int.): 426 $[M]^+$ (4.1), 411 $[M-15]^+$ (10), 393 $[M-15-18]^+$ (7), 355 (1), 342 (1), 339 (1), 325 (1), 229 (5), 207 (10), 205 (3), 83 (5), 69 (100), 41 (56).

Oxidation of compound **2**. Compound **2** (35 mg) was dissolved in 3 ml Me_2CO, and 0.5 ml Jones reagent were added at room temp. After shaking for 5 min, the soln was diluted with water and extracted with $CHCl_3$. The reaction product was recrystallized from EtOH to afford 29.8 mg of **3**, glassy solid; $[\alpha]_D$ +17.15° ($CHCl_3$; *c* 0.054); IR ν_{max} cm^{-1}: 3940, 1715, 1450, 1390, 1360; ^1H NMR (300 MHz, $CDCl_3$): δ 0.75 (3H, *s*, H-30), 0.85 (3H, *d*, *J* = 6 Hz, H-21), 0.89 (3H, *s*, H-18), 1.04 (3H, *s*, H-19). 1.05 (3H, *s*, H-28), 1.10 (3H, *s*, H-29), 1.60, 1.68 (each 3H, *s*, H-26 and H-27, respectively), 5.09 (1H, *t*, *J* = 7.3 Hz, H-24); CD (MeOH; *c* 0.05) $\Delta\varepsilon$ (nm): +0.06 (260), +0.168 (270), +0.271 (280), +0.310 (287), +0.1811 (300), 0 (308), −0.06 (320).

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