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DITERPENES FROM *STEVIA MONARDAEFOLIA**

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Key Word Index—*Stevia monardaefolia*; Compositae; Eupatorieae; labane-type diterpenes; 6α-angeloyloxy nidorellol; 6α-angeloyloxy sclareol.

Abstract—An investigation of *Stevia monardaefolia* resulted in the isolation of two new labdane type diterpenes, 6α-angeloyloxy nidorellol and 6α-angeloyloxy sclareol. Their structures were established by chemical and spectroscopic methods.

INTRODUCTION

In continuation of our chemical systematic studies of plants of the tribe Eupatorieae (Compositae) [1, 2], we have undertaken a study of *Stevia monardaefolia* and isolated two new labdane type diterpenes which were shown to be 6α-angeloyloxy nidorellol (**1a**) and 6α-angeloyloxy sclareol (**2**), in addition to the known kaurenoic acid, angeloylgrandifloric acid, a mixture of sitosterol and stigmasterol and 2,2-dimethyl-7-methoxy chromene and 2,2-dimethyl-7, 8-dimethoxy chromene which we have also isolated from *Eupatorium aschembornianum* (unpublished observation).

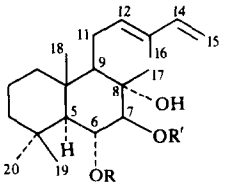
RESULTS AND DISCUSSION

6α-Angeloyloxy nidorellol (**1a**), $C_{25}H_{40}O_4$ $[M]^+$ at *m/z* 404), $[\alpha]_D$ −34.8°, was isolated as a crystalline compound, mp 114–115°, which was shown to be a labdane type with a conjugated diene system in the side chain, which showed UV absorption at 233 nm (ε 21 980) and IR absorption at 1645, 1600, 985, 890 cm^{-1}. The ^1H NMR spectrum displayed an AMX pattern characteristic of a vinyl group with signals at δ 4.9 (*br d*, J = 10 Hz), 5.05 (*br d*, J = 17 Hz) and a doublet of doublets at 6.37 (J = 17 Hz, J = 10 Hz) due to H-14. The chemical shift of the latter proton indicated that the Δ^{12} double bond had the *trans*-configuration [3, 4]. The presence of an angelate side chain ester (1710 cm^{-1}) was indicated by the mass spectral ion peaks at *m/z* 304 $[M-C_5H_8O_2]^+$, 83 $[C_5H_7O]^+$, (100.0%) and 55 $[C_4H_7]^+$ characteristic of this type of ester [5], and the typical vinyl proton quartet at δ 6.06 [6] and the vinyl methyl group multiplets at δ 1.92 and 1.98 in the ^1H NMR spectrum.

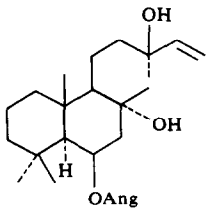
The ^1H NMR spectrum (Table 1) of **1a** also showed the five methyl group signals of the labdane skeleton, a vinyl one at δ 1.76 and four tertiary ones at 1.21 (3H), 0.98 (6H) and 0.90 (3H). A broad triplet at 5.58 (J = 7 Hz) was assigned to the vinyl proton at C-12 and a doublet of doublets at 5.27 (J = 10 Hz, J = 10 Hz) was due to H-6 since this signal was shifted upfield to δ 3.6 after hydrolysis. Finally a doublet at 3.53 (J = 10 Hz) was assigned to the proton (H-7) on the carbon bearing a secondary hydroxyl group since this signal shifted downfield to δ 4.91 upon acetylation.

Acetylation of **1a** with Ac_2O-pyridine gave the monoacetate (**1b**) (1735 cm^{-1}; δ 1.97 *s*, 3H) whose IR spectrum exhibited the presence of a hydroxyl group (3490 cm^{-1}) indicating that **1a** had one tertiary and one secondary hydroxyl group.

Catalytic hydrogenation of **1a** afforded a tetrahydro derivative **3**, $C_{25}H_{44}O_4$ $[M]^+$ at *m/z* 408), whose ^1H NMR spectrum clearly showed the signal due to the methyne protons bearing the side chain ester and the



1a R = Ang R′ = H
1b R = Ang R′ = Ac
1c R = H R′ = H
Ang = Angeloyl



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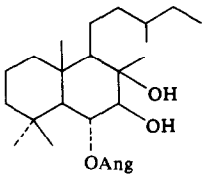

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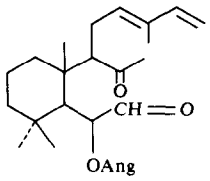

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Table 1. ^{1}H NMR spectral data of 6α-angeloyloxy nidorellol (**1a**), 6α-angeloyloxy sclareol (**2**) and derivatives*

	1a	1b	1c	2	3	4	5a	5c	5d	6
H-6	5.27 *dd*	†	3.6 *dd*	†	5.26 *dd*	5.68 *brs*	5.23 *dt*	3.70 *dt*	—	—
H-7	3.53 *d*	4.91 *d*	3.37 *d*	—	3.47 *d*	9.61 *s*	—	2.04 *dd*	2.67 *d* 2.43 *d*	5.76 *brs*
H-12	5.58 *brt*	†	5.53 *brt*	—	—	5.29 *brt*	—	—	—	—
H-14	6.37 *dd*	6.30 *dd*	6.33 *dd*	5.9 *dd*	—	6.29 *dd*	—	—	—	—
H-15 *cis*	4.9 *brd*	4.88 *brd*	4.89 *brd*	5.05 *dd*	—	4.91 *brd*	—	—	—	—
H-15 *trans*	5.05 *brd*	5.02 *brd*	5.03 *brd*	5.19 *dd*	—	5.06 *brd*	—	—	—	—
H-16	1.76 *brs*	1.75 *brs*	1.75 *brs*	1.26 *s*	0.92 *d*	1.72 *brs*	1.14 *s*	1.15 *s*	1.18 *s*	1.18 *s*
H-17	1.21 *s*	1.22 *s*	1.17 *s*	1.26 *s*	1.20 *s*	2.1 *s*	1.28 *s*	1.15 *s*	1.18 *s*	1.92 *s*
H-18	0.90 *s*	0.90 *s*	0.9 *s*	0.85 *s*	0.89 *s*	0.89 *s*	0.87 *s*	0.80 *s*	0.80 *s*	0.87 *s*
H-20	0.98 *s*	0.93 *s*	1.0 *s*	0.87 *s*	0.91 *s*	1.06 *s*	0.92 *s*	0.96 *s*	0.94 *s*	1.12 *s*
H-19	0.98 *s*	1.0 *s*	1.17 *s*	1.0 *s*	0.95 *s*	1.27 *s*	1.0 *s*	1.08 *s*	1.18 *s*	1.16 *s*
Ang	6.06 *brq*	†	—	6.05 *brq*	6.06 *brq*	†	6.01 *brq*	—	—	—
Ac	—	1.97 *s*	—	—	—	—	—	—	—	—

*Run at 100 MHz in $CDCl_3$ with TMS as int. standard; **5c** was run in DMSO-d_6. Values are in ppm (δ).

†Signal obscured. $J_{(5\alpha,6\beta)} = J_{6\beta,7\alpha} = 10$ Hz; $J_{(6\beta,7\beta)} = 4$ Hz; $J_{(7\alpha,7\beta)} = 12$ Hz; $J_{(11,12)} = 7$ Hz; $J_{(14,15cis)} = 10$ Hz; $J_{(14,15\,trans)} = 17$ Hz; $J_{(15\,cis\,15\,trans)} = 2$ Hz.

secondary hydroxyl group, as a triplet at δ 5.26 (J = 10 Hz) and a doublet at 3.47 (J = 10 Hz), indicating that these groups could be placed at C-6 and C-7 respectively. Therefore the tertiary hydroxyl group must be placed at C-8. Furthermore the coupling constants indicated a *trans*-diaxial relationship between H-5, H-6 and H-7. Confirmation of the relative position of the hydroxyl groups was achieved by periodic acid oxidation of **1a** to afford the keto-aldehyde **4** $[M]^+$ at m/z 402) with IR absorption at 1700, 1730 cm^{-1} (δ 2.1 *s*, 3H; 9.61 *s*, 1H).

Alkaline hydrolisis of **1a** with sodium methoxide furnished the triol **1c**, whose ^{1}H NMR spectral data were very similar to those of austroinulin isolated from *Austroeupatorium inulaefolium* [7].

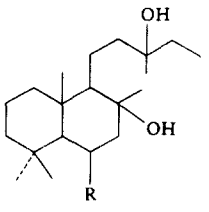
The optical rotation value $[\alpha]_D$ −31.9° of the triol **1c**, led to the suggestion that **1a** might have the structure antipodal to austroinulin [7], $[\alpha]_D$ +24.0°, as long as the stereochemistry of the Δ^{12} double bond on the side chain has little effect on the optical rotation value of the bicyclic nucleus, as can be observed from the *cis*- and *trans*-biformene [4], *cis*- and *trans*-communic acid [3, 9], and *cis*- and *trans*-ozic acid [10].

Finally, comparison of the ^{1}H NMR and IR spectra of 6α-hydroxy nidorellol recently isolated from *Nidorella auriculata* ssp. *polycephala* [8], with those of the triol **1c** indicated them to be identical.

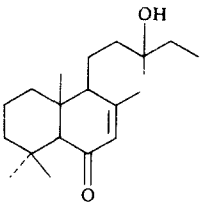
6α-Angeloyloxy sclareol (**2**), $C_{25}H_{42}O_4$ $[M]^+$ at m/z 406), $[\alpha]_D$ −26.4°, was a crystalline labdane-type diterpene with an α, β-unsaturated ester (1710 cm^{-1}), which was shown to be an angelate as in the case of 6α-angeloyloxy nidorellol (**1a**). The IR and ^{1}H NMR spectra indicated the presence of tertiary hydroxyl groups (3340, 1150 cm^{-1}) and five tertiary methyl groups, two of them located on carbons bearing a hydroxyl group (δ 1.26, *s*, 6H) and a vinyl group. The angelic ester and a hydroxyl group were placed at C-6 and C-8, respectively, based on the following evidence.

Ozonolysis of the dihydro derivative **5a** resulted in the pyruvate derivative **5b** (1720 cm^{-1}; δ 2.41 *s*, 3H). Alkaline hydrolisis of **5b** afforded the triol **5c**, which was oxidized to the ketone **5d**. Dehydration of **5d** with magnesium sulfate gave the α,β-unsaturated diketone **6** (1670 cm^{-1}; UV λ_{max} 240 nm)[11].

Finally the position of the second hydroxyl group was determined by dehydration of **2** with magnesium sulfate resulting in the *cis–trans* mixture of compounds **7** and **7a**, whose ^{1}H NMR spectrum clearly showed the H-14 signal for the *trans*-isomer **7** at δ 6.35 and for the *cis*-isomer **7a** at 6.85. These chem-



5a R = OAng
5b R = OCOCOMe
5c R = OH
5d R = O



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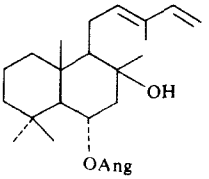

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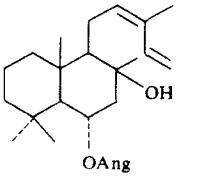

7a

ical shifts were in good agreement with those of *cis*- and *trans*-communic acid as well as *cis*- and *trans*-biformene [3, 4].

Based on all these facts we propose the structures **1a** and **2** as the more likely structures for the new isolated diterpenes.

EXPERIMENTAL

Stevia monardaefolia H. B. K. was collected in December 1978, 35 km south of the México–Cuernavaca road, D. F. Calderón 16. Voucher is on deposit at Herbarium of Instituto de Biología (UNAM,), México. Dried leaves, flowers and stems (1250 g) were extracted with petrol (12 l.); the extract was processed as previously described [1, 2] providing 21.0 g crude syrup which was chromatographed on a column packed with Si gel (1000 g) and eluted with petrol and mixtures of petrol–C_6H_6–EtOAc. From the low polar fractions 2,2-dimethyl-7-methoxychromene and 2,2-dimethyl-7, 8-dimethoxychromene were isolated in addition to the known compounds sitosterol, stigmasterol, kaurenoic acid and its corresponding 15 α-angeloyloxy derivative (angeloylgrandifloric acid) which was identified by comparison with an authentic sample.

6α-Angeloyloxy nidorellol (**1a**). Chromatography fractions eluted with petrol–C_6H_6–EtOAc (3 : 6 : 1) provided after purification by TLC 1.9 g **1a** as a crystalline compound, mp 114–115°. $[\alpha]_D$ −34.8° ($CHCl_3$); UV λ_{max}^{EtOH} nm: 233 (ϵ 21980); IR ν_{max}^{Film} cm^{-1}: 3430, 1710, 1640, 1600, 1150; MS *m/z*: 404 $[M]^+(C_{25}H_{40}O_4)$, 386 $[M-H_2O]^+$, 304 $[M-C_5H_6O_2]^+$, 286 $[M-C_5H_80_2-H_2O]^+$, 83 $[C_5H_7O]^+$(100.0%), 55 $[C_4H_7]^+$.

6α-Angeloyloxy sclareol (**2**). The chromatography fraction eluted with petrol–C_6H_6–EtOAc (1 : 7 : 2) provided after TLC purification 1.5 g **2** as a crystalline material mp 109–110°. $[\alpha]_D$ −26.4° ($CHCl_3$); IR ν_{max}^{Film} cm^{-1}: 3400, 1710, 1650, 1150. MS *m/z*: no $[M]^+$, 388 $[M-H_2O]^+$, 288 $[M-C_5H_8O_2-H_2O]^+$, 83 $[C_5H_7O]^+$, 55 $[C_4H_7]^+$, 43 $[C_3H_7]^+$(100.0%).

Hydrogenation of **1a**. Compound **1a** (80 mg) was hydrogenated using PtO_2 as catalyst yielding 40 mg the tetrahydro derivative **3**. $[\alpha]_D$ −5.6° ($CHCl_3$), UV λ_{max}^{EtOH} nm: 244 (ϵ 4610). IR ν_{max}^{Film} cm^{-1}: 3450, 1715, 1640, 1150. MS *m/z*: 408 $[M]^+$ $(C_{25}H_{44}O_4)$, 390 $[M-H_2O]^+$, 308 $[M-C_5H_8O_2]^+$, 83 $[C_5H_7O]^+$ (100.0%), 55 $[C_4H_7]^+$.

Acetylation of **1a**. Acetylation of **1a** (111 mg) with Ac_2O–pyridine gave after TLC purification the oily monoacetate **1b**. $[\alpha]_D$ −36.7° ($CHCl_3$), IR ν_{max}^{Film} cm^{-1}: 3490, 1735, 1710, 1640, 1600, 1150. MS *m/z*: 446 $[M]^+$ $(C_{25}H_{42}O_5)$, 428 $[M-H_2O]^+$, 328 $[M-C_5H_8O_2-H_2O]^+$, 386 $[M-AcOH]^+$, 286 $[M-C_5H_8O_2-H_2O-AcOH]^+$, 83 (100%), 55, 43.

Hydrolysis of **1a**. A 200 mg sample of **1a** was treated with NaOMe in MeOH (10 ml), the reaction being monitored by TLC. After 2 hr the reaction was worked-up and the residue purified by TLC, yielding 77 mg the triol **1c** as a colourless oil. $[\alpha]_D$ −31.9°. UV λ_{max}^{EtOH} nm: 236 (ϵ 16, 150), IR ν_{max}^{Film} cm^{-1}: 3400, 1640, 1600, 1100. MS *m/z*: no $[M]^+$, 304 $[M-H_2O]^+$, 286 $[M-2H_2O]^+$, 271 $[M-2H_2O-Me]^+$, 43 (100.0%).

Oxidation of **1a**. Periodic acid (100 mg) was stirred with 20 ml Et_2O for 1 hr, and decanted over a soln of 125 mg of **1a** in Et_2O, the reaction being monitored by TLC. When the reaction was completed the ethereal soln was washed with H_2O, dried and the solvent evaporated yielding 30 mg **4** after TLC purification. $[\alpha]_D$ +34.5°, UV λ_{max}^{EtOH} nm: 232 (ϵ 20100), IR ν_{max}^{Film} cm^{-1}: 1730, 1710, 1700. MS *m/z*: 402 $[M]^+(C_{25}H_{38}O_4)$, 302 $[M-C_5H_8O_2]^+$, 259 $[302-MeCO]^+$, 137 $[C_{10}H_{17}]^+$, 83 (100.0%), 55, 43.

Hydrogenation of **2**. 100 mg **2** in EtOH was hydrogenated using Pt_2O (10 mg) as catalyst. After elimination of the solvent 40 mg **5a** were obtained as a colourless oil. $[\alpha]_D$ −21.3°, IR ν_{max}^{film} cm^{-1}: 3350, 1710, 1650, 1150. MS *m/z*: no $[M]^+$, 390 $[M-H_2O-C_5H_8O_2]^+$, 83, 55 (100.0%), 43.

Dehydration of **2**. Dry $MgSO_4$ (1.5 g) was added to a soln of 150 mg **2** in C_6H_6 and refluxed for 20 hr, the reaction being monitored by TLC. When the reaction was completed the $MgSO_4$ was filtered and the solvent evaporated yielding 47 mg of a mixture of **7** and **7a** after TLC purification. UV λ_{max}^{EtOH} nm: 225 (ϵ 19190). IR ν_{max}^{film} cm^{-1}: 3590, 1710, 1640, 1630, 1150. MS *m/z*; no $[M]^+$, 370 $[M-H_2O]^+$, 288 $[M-C_5H_8O_2]^+$, 270 $[M-H_2O-C_5H_8O_2]^+$, 83 (100.0%), 55, 43.

Pyruvate (**5b**). A soln of 235 mg **5a** in CS_2 was ozonized for 5 min. The ozonide was decomposed with triphenylphosphine, the solvent removed and the residue purified by TLC giving 34 mg of **5b**. IR ν_{max}^{film} cm^{-1}: 3370, 1720, 1140. MS *m/z*: no $[M]^+$, 378 $[M-H_2O]^+$, 291 $[M-H_2O-MeCOCO_2]^+$, 273 $[M-2H_2O-MeCOCO_2]^+$, 43 (100.0%).

Hydrolysis of **5b**. A soln of 35 mg **5b** in 20 ml MeOH was treated with 45 mg K_2CO_3 at room temp. After 3 hr, the reaction mixture was worked-up to give **5c**, mp 167–169° (Me_2CO), $[\alpha]_D$ −15.0°, IR ν_{max}^{KBr} cm^{-1}: 3380, 1150. MS *m/z*: 308 $[M-H_2O]^+$, 290 $[M-2H_2O]^+$, 275 $[M-2H_2O-Me]^+$, 18 (100%).

Oxidation of **5a**. Pyridinium dichromate (36 mg) was added to a soln of 40 mg **5c** in 25 ml CH_2Cl_2 and stirred for 1 hr, then diluted with Et_2O, filtered, concd and the reaction mixture purified by TLC to give **5d** mp 108–110°, $[\alpha]_D^{25}$ −22.3°, IR ν_{max}^{film} cm^{-1}: 3370, 1700, 1140. MS *m/z*: 306 $[M-H_2O]^+$, 291 $[M-H_2O-Me]^+$, 43 (100%).

Dehydration of **5d**. 30 mg **5d** in 25 ml C_6H_6 was treated with 300 mg dry $MgSO_4$ as described before to yield 12 mg **6** as a colourless oil. $[\alpha]_D^{25}$ −23.1°, UV λ_{max}^{EtOH} nm: 240 (ϵ 9000), IR ν_{max}^{film} cm^{-1}: 3420, 1670, 1630, 1150. MS *m/z*: 306 $[M]^+(C_{20}H_{34}O_2)$, 288 $[M-H_2O]^+$, 135 (100%).

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