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ANDESGENIN, A NEW STEROID SAPOGENIN FROM *SOLANUM HYPOMALACOPHYLLUM**

ANTONIO G. GONZÁLEZ, COSME G. FRANCISCO, RAIMUNDO FREIRE, ROSENDO HERNÁNDEZ, JOSÉ A. SALAZAR and ERNESTO SUÁREZ

Department of Organic and Biochemistry, University of La Laguna, Instituto de Investigaciones Químicas, C.S.I.C., Tenerife, Spain

and

ANTONIO MORALES and ALFREDO USUBILLAGA

Instituto de Investigación Química, Facultad de Farmacia, Universidad de Los Andes, Mérida, Venezuela

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Abstract—The structure of andesgenin, a new steroid sapogenin isolated from *Solanum hypomalacophyllum*, was determined on the basis of spectral data and synthesis of its Huang–Minlon reduction products to be (20 *S*, 22 *R*, 25 *R*)-3β-hydroxy-5α-cholestan-22,26-epoxy-4,23-dione.

INTRODUCTION

From *Solanum hypomalacophyllum* Bitter, a tree native to the Venezuelan Andes, the 2 steroid alkaloids solaphyllidine [1] and solamaladine [2] were obtained. The present work reports the structure and stereochemistry of andesgenin (**1a**), a neutral sapogenin isolated from the acid-hydrolyzed juice of the green berries of this plant. By synthesis of its Huang–Minlon reduction products (**1c**) and (**2a**) the side-chain stereochemistry in (**1a**) was determined as 20 *S*, 22 *R*, 25 *R*.

RESULTS AND DISCUSSION

Andesgenin (**1a**) ($C_{27}H_{42}O_4$, M^+ 430·3074) presented IR absorptions for hydroxyl and carbonyl groups (3495, 1712 cm^{-1}). In its MS the base peak at *m/e* 114·0651 corresponded to fragment [**a**] ($C_6H_{10}O_2$), which originated by a McLafferty rearrangement of the C-23 carbonyl, and the prominent ion at *m/e* 85·0657 to [**a**] − COH. The NMR spectrum showed a multiplet at δ 4·02 (2H, $W_{1/2}$ 21 Hz) attributed to the C-3 and the equatorial C-26 protons, a 1-proton doublet at δ 3·67 (*J* 2 Hz, C-22), and a double doublet at δ 3·23 of the axial C-26 proton.

Mild acetylation of (**1a**) gave (**1b**) ($C_{29}H_{44}O_5$). Its NMR spectrum showed the C-3 proton as a multiplet at δ 5·1 ($W_{1/2}$ = 22 Hz) which in C_6D_6 appeared as a double doublet centred at δ 5·17 (AB*X* system, $J_{AX} + J_{BX}$ 20 Hz). The shape of the 2 signals corresponding to the C-26 protons (see Experimental) was only compatible with an equatorial methyl at C-25. The chemical shift of the C-10 Me in (**1a**) and (**1b**) (δ 0·71 and 0·76 respectively) was indicative of a 3β-hydroxy-4-keto-5α-androstane moiety [1,2]. The MS of (**1b**) (M^+ 472) displayed the same fragments [**a**] and [**a**] − COH as that of the alcohol and confirmed the position of the hydroxyl in (**1a**) at C-3.

Huang–Minlon reduction of (**1b**) gave (**1c**) and (**2a**). The IR spectrum of the former ($C_{27}H_{46}O$, M^+ 386) showed the absence of carbonyl and acetate groups and in its MS the base peak appeared at *m/e* 99 which corresponds to fragment [**b**]

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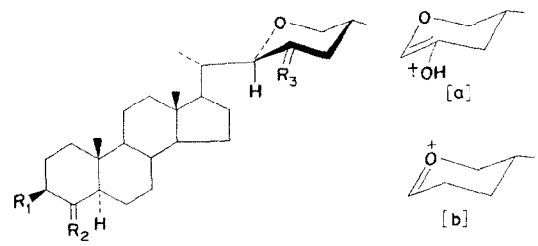
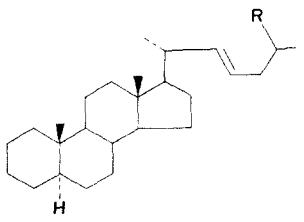


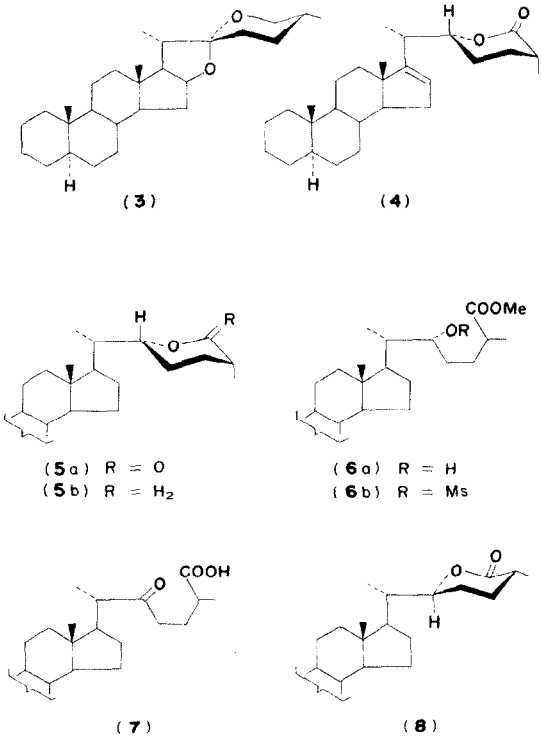
(1a) R_1 = OH R_2 = O R_3 = O
(1b) R_1 = OAc R_2 = O R_3 = O
(1c) R_1 = H R_2 = H_2 R_3 = H_2



(2a) R = CH_2OH
(2b) R = COOMe

formed by cleavage of the C-22, C-23 bond. The NMR signals of the C-22 and C-26 protons were only compatible with structure (**1c**). Compound (**2a**) ($C_{27}H_{46}O$) has a primary hydroxyl at C-26 and a *trans*-disubstituted double bond [3] as was inferred from its IR and NMR data. The formation of (**1c**) proved that andesgenin possesses an α-hydroxyketo group [4] in ring A. On the other hand, comparison of the fragments [**a**] and [**b**] which appear in the MS of (**1a**) and (**1c**) respectively, along with the formation of the Huang–Minlon reduction product (**2a**) allowed the location of the side-chain carbonyl in (**1a**) at C-23 [5].

In order to determine the side-chain stereochemistry of andesgenin (**1a**) we synthesized (**1c**) and (**2a**) from compound (**4**), which in turn was obtained from (25 *R*)-5α-spirostan (**3**) [6]. Hydrogenation of (**4**) in EtOH over 10% Pd-C gave (**5a**) whose structure was established on the basis of IR and NMR data [1730 cm^{-1} (δ-lactone); δ 4·3 (1H, *m*, $W_{1/2}$ 16 Hz, C-22)]. Subsequent methanolysis with $NaHCO_3$ in MeOH yielded (**6a**) which was mesylated to give (**6b**) and then refluxed in DMF to give (**2b**) as the major product. This was reduced with $LiAlH_4$ to yield (**2a**), identical in all respects with the compound obtained by Huang–Minlon reduction of (**1b**). Hence, the stereochemistry at C-20 and C-25 in (**1a**) must be the same as in (**3**) (20 *S*, 25 *R*). Reduction of (**5a**) with



(3) (4)

(5a) R = O
(5b) R = H_2

(6a) R = H
(6b) R = Ms

(7) (8)

$NaBH_4$–BF_3 etherate in THF-diglyme [7] yielded (**5b**). Its MS fragmentation pattern coincided with that of (**1c**) but their IR and NMR spectra differed considerably. The shape of the multiplet at δ 3·64 ($W_{1/2}$ 3 Hz) corresponding to the two C-26 protons of (**5b**) was only compatible with an axial methyl at C-25. Since (**1c**) and (**5b**) have the *R* configuration at C-25, the sole possibility that the Me group at this carbon atom be equatorial in (**1c**) and axial in (**5b**) is that both compounds are epimers at C-22.

On the other hand, Jones oxidation of (**6a**) gave the ketoacid (**7**), which upon reduction with $NaBH_4$ in MeOH–THF, afforded the lactones (**5a**) and (**8**). The latter compound was reduced with $NaBH_4$–BF_3 etherate [7] to produce (**1c**) and this compound proved to be identical with the Huang–Minlon reduction product obtained previously. The structure of andesgenin therefore corresponds to (20 *S*, 22 *R*, 25 *R*)-3β-hydroxy-5α-cholestan-22,26-epoxy-4,23-dione (**1a**).

EXPERIMENTAL

Mp's, determined on a Kofler hot-stage apparatus, are uncorr. Optical rotations were measured in $CHCl_3$ and NMR spectra in $CDCl_3$ with TMS as internal reference. Dry column chromatography was carried out on Si gel 0·063–0·20 mm.

Isolation of andesgenin. To juice (15 l.) obtained from green berries of *Solanum hypomalacophyllum*, which were collected at Páramo del Batallón near Delgadito (Mérida, Venezuela) conc H_2SO_4 was added till it was 2 N. After refluxing for 4 hr, it was left overnight. The ppt. was filtered and extracted with Me_2CO. Dry column chromatography (C_6H_6–EtOAc, 9:1) of the extract gave andesgenin (1·5 g).

Andesgenin (**1a**). Mp 211–214° (Me_2CO), $[\alpha]_D$ +39° (*c* 0·254). (Found: C, 75·30; H, 9·78. $C_{27}H_{42}O_4$ requires: C, 75·31; H, 9·83%). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3495 (OH), 1712 (CO). MS (probe) 70 eV *m/e* (rel. int.): 430·3074 M^+ (4), 415·2877 (M^+ − Me; 1), 397·2734 (M^+ − H_2O − Me; 2), 317·2469 (M^+ − $C_6H_9O_2$; 8), 114·0651 ([**a**]; 100), 85·0657 ([**a**] − COH; 97). NMR: δ 4·02 (2H, *m*, $W_{1/2}$ 21 Hz, C-3, eq. C-26), 3·67 (1H, *d*, *J* 2 Hz, C-22), 3·23 (1H, *dd*, ax. C-26), 0·92 (3H, *d*, *J* 7 Hz, C-21 or C-27), 0·86 (3H, *d*, *J* 7 Hz, C-27 or C-21), 0·71 (3H, *s*, C-19), 0·68 (3H, *s*, C-18). *Acetate* (**1b**), prepared from (**1a**) as usual, mp 239–241° (MeOH), $[\alpha]_D$ +2° (*c* 0·256). (Found: C, 73·99; H, 9·82. $C_{29}H_{44}O_5$ requires: C, 73·69; H, 9·38%.) IR ν_{max}^{KBr} cm^{-1}: 1745, 1240 (OAc), 1730 (CO). MS (probe) 70 eV *m/e* (rel. int.): 472 M^+ (4), 412 (M^+ − HOAc; 2), 359 (M^+ − $C_6H_9O_2$; 11), 299 (M^+ − $C_6H_9O_2$ − HOAc; 17), 114 ([**a**]; 95), 85 ([**a**] − COH; 100). NMR: δ 5·1 (1H, *m*, $W_{1/2}$ 22 Hz, C-3), 4·01 (1H *m*, $W_{1/2}$ 20 Hz, eq. C-26), 3·66 (1H, *d*, *J* 2 Hz, C-22), 3·20 (1H, *dd*, ax. C-26), 2·06 (3H, *s*, OAc), 0·92 (3H, *d*, *J* 7 Hz, C-21 or C-27), 0·86 (3H, *d*, *J* 7 Hz, C-27 or C-21), 0·76 (3H, *s*, C-19), 0·68 (3H, *s*, C-18).

Compounds (**1c**) *and* (**2a**) *from* (**1b**). A soln of (**1b**) (100 mg) in diethyleneglycol (2·5 ml) was refluxed with $N_2H_4.H_2O$ (0·2 ml) for 1·5 hr. KOH (140 mg) was added, the H_2O and excess $N_2H_4.H_2O$ were distilled off and the soln refluxed again for 1·5 hr. It was poured into H_2O, acidified with dil HCl and extracted with $CHCl_3$. Dry column chromatography of the residue with C_6H_6–petrol (1:4) and C_6H_6 gave (**1c**) (41 mg) and (**2a**) (26 mg). (**1c**), mp 155–157° (Me_2CO), $[\alpha]_D$ − 3° (*c* 0·302). (Found: C, 83·86; H, 11·70. $C_{27}H_{46}O$ requires: C, 83·87; H, 11·99%.) MS (probe) 70 eV *m/e* (rel. int.): 386 M^+ (0·5), 371 (M^+ − Me; 0·5), 286 (M^+ − $C_6H_{12}O$; 0·5), 271 (M^+ − $C_6H_{12}O$ − Me; 0·5), 258 (M^+ − $C_8H_{16}O$; 6), 243 (M^+ − $C_6H_{16}O$ − Me; 0·5), 99 ([**b**]; 100). NMR: δ 3·20 (1H, *m*, $W_{1/2}$ 20 Hz, C-22), 3·84 (1H, *m*, $W_{1/2}$ 18 Hz, eq. C-26), 2·88 (1H, *dd*, ax. C-26), 0·74 (3H, *s*, C-19), 0·61 (3H, *s*, C-18). (**2a**), mp 99–101° (MeOH), $[\alpha]_D$ +9° (*c* 0·242). (Found: C, 83·65; H, 12·17. $C_{27}H_{46}O$ requires: C, 83·87; H, 11·99%.) IR $\nu_{max}^{CS_2}$ cm^{-1}: 3610 (OH). 970 (*trans* R–CH=CH–R'). NMR: δ 5·30 (2H, *m*, $W_{1/2}$ 10 Hz, *trans* R–CH=CH–R'), 3·47 (2H, *d*, *J* 6 Hz, C-26), 0·76 (3H, *s*, C-19), 0·64 (3H, *s*, C-18).

Hydrogenation of (**4**). (**4**) (1·04 g) dissolved in abs EtOH (200 ml) was hydrogenated over 10% Pd-C (500 mg) at room temp. and atm pres for 2 hr. Dry column chromatography (C_6H_6–EtOAc, 19:1) gave (**5a**) (900 mg), mp 222–225° (C_6H_6–EtOAc), $[\alpha]_D$ + 4° (*c* 0·276). IR ν_{max}^{KBr} cm^{-1}: 1730 (δ-lactone). NMR: δ 4·3 (1H, *m*, $W_{1/2}$ 16 Hz, C-22), 1·19 (3H, *d*, *J* 7 Hz, C-27), 0·94 (3H, *d*, *J* 7 Hz, C-21), 0·76 (3H, *s*, C-19), 0·66 (3H, *s*, C-18). (**2a**) *from* (**5a**). (**5a**) (600 mg) was stirred with a satd soln (50 ml) of $NaHCO_3$ in MeOH at room temp. for 12 hr. Usual work-up and chromatographic separation (C_6H_6–EtOAc, 19:1) yielded (**6a**) (250 mg) besides starting material (300 mg). Mesylation of (**6a**) with MsCl in Py gave (**6b**) which without further purification was refluxed in DMF (5 ml) for 30 min. Chromatography on $AgNO_3$–Si gel (1:4) with C_6H_6–petrol (1:4) gave (**2b**) (140 mg), mp 86–89° (MeOH), $[\alpha]_D$ −1° (*c* 0·222). (Found: C, 81·36; H, 10·90. $C_{28}H_{46}O_2$ requires: C, 81·10; H, 11·18%.) IR ν_{max}^{KBr} cm^{-1}: 1740 (COOMe), 975 (*trans* R–CH=CH–R'). NMR: δ 5·25 (2H, *m*, $W_{1/2}$ 12 Hz, *trans* R–CH=CH–R'), 3·63 (3H, *s*, COOMe), 1·09 (3H, *d*, *J* 7 Hz, C-27), 0·95 (3H, *d*, *J* 7 Hz, C-21), 0·75 (3H, *s*, C-19), 0·62 (3H, *s*, C-18). To a soln of (**2b**) (46 mg) in Et_2O (10 ml) excess $LiAlH_4$ was added and the mixture refluxed for 30 min. Usual work-up gave (**2a**) (36 mg), identical with the Huang–Minlon reduction product obtained above (mmp, TLC, IR and NMR spectra superimposable).

(**5b**) *from* (**5a**). To a soln of (**5a**) (50 mg) in THF (3·5 ml) BF_3 etherate (0·5 ml) and $NaBH_4$ (18 mg) were added and the mixture left till bubbling ceased. After adding diglyme (1·5 ml) the soln was kept at room temp. for 30 min and then refluxed for 2 hr. It was poured into H_2O, extracted with $CHCl_3$ and the organic layer was washed with satd $NaHCO_3$. Chromatography (C_6H_6–petrol, 1:1) yielded (**5b**) (33 mg), mp 162–165° (Me_2CO). (Found: C, 83·76; H, 12·22. $C_{27}H_{46}O$ requires: C, 83·87; H, 11·99%.) MS (probe) 70 eV *m/e* (rel. int.): 386 M^+ (0·5), 371 (M^+ − Me; 0·5), 286 (M^+ − $C_6H_{12}O$; 1), 271 (M^+ − $C_6H_{12}O$ − Me; 0·5), 258 (M^+ − $C_8H_{16}O$; 6), 243 (M^+ − $C_8H_{16}O$ − Me; 0·5), 99 ([**b**]; 100). NMR: δ 3·64 (2H, *m*, $W_{1/2}$ 3 Hz, C-26), 3·25 (1H, *m*, $W_{1/2}$ 16 Hz, C-22), 1·06 (3H, *d*, *J* 7 Hz, C-27 or C-21), 0·94 (3H, *d*, *J* 7 Hz, C-21 or C-27), 0·76 (3H, *s*, C-19), 0·65 (3H, *s*, C-18).

(**8**) *from* (**5a**). A soln of (**5a**) (200 mg) in Me_2CO (10 ml) was treated with Jones reagent till the reaction was completed. Usual work-up gave a residue consisting mainly of (**7**) which without purification was dissolved in EtOH–THF, 1:6 (35 ml). After adding excess $NaBH_4$ the soln was stirred at room temp. for 6 hr, poured into H_2O, acidified and extracted with $CHCl_3$. Chromatography (C_6H_6) yielded, besides (**5a**) (42 mg), (**8**) (51 mg), mp 191–193° (Me_2CO), $[\alpha]_D$ +22° (*c* 0·204). (Found: C, 80·44; H, 11·58. $C_{27}H_{44}O_2$ requires: C, 80·74; H, 11·37%.) IR ν_{max}^{KBr} cm^{-1}: 1730 (δ-lactone). NMR: δ 4·38 (1H, *m*, $W_{1/2}$ 16 Hz, C-22), 2·26 (3H, *d*, *J* 7 Hz, C-27), 0·74 (3H, *s*, C-19), 0·62 (3H, *s*, C-18).

(**1c**) *from* (**8**). A soln of (**8**) (60 mg) in THF (4 ml) was treated with BF_3 etherate (1 ml), $NaBH_4$ (50 mg) and diglyme (4 ml) as described above for (**5b**) synthesis. Chromatography (C_6H_6–petrol, 1:1) gave (**1c**) (29 mg) which was identical with the Huang–Minlon reduction product obtained above (mmp, TLC, IR and NMR spectra superimposable).

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