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STEROIDAL SAPOGENINS FROM *SOLANUM SCORPIOIDEUM*

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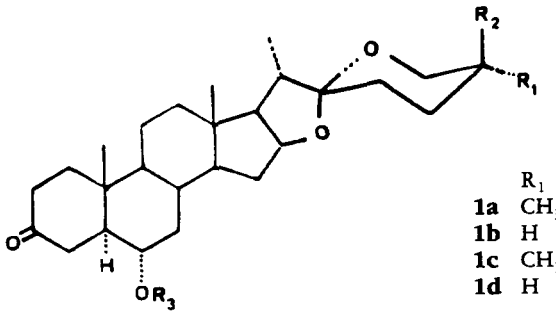
ABSTRACT.—Two new ketosapogenins, (22*R*,25*R*)-6α-hydroxy-5α-spirostan-3-one [**1a**] and its 25*S* epimer [**1b**] were isolated from a $CHCl_3$ extract of the juice obtained from the green berries of *Solanum scorpioideum*. Chlorogenin [**2a**] and neochlorogenin [**2b**] were also isolated.

Solanum scorpioideum Rusby (Solanaceae) is a small tree native to the Columbian Andes. Perez Medina *et al.* (1), in a screening of 60 Columbian Solanaceae, reported a negative test for steroidal glycoalkaloids in the fruits of this plant. The juice obtained from the green berries was extracted with $CHCl_3$. Column chromatography of this extract yielded **1a**, mp 208-211°, $[\alpha]D^{21.5}-29.6$ (*c* 1.98, $CHCl_3$) **1b**, mp 215-219°, $[\alpha]D^{21.5}-52.7$ (*c* 4.21, $CHCl_3$) and small amounts of chlorogenin [**2a**] and neochlorogenin [**2b**].

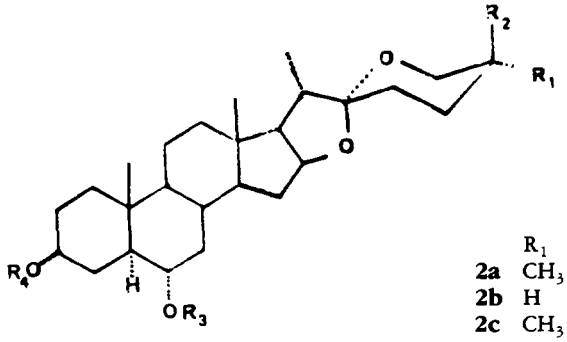
Mass spectra and elementary analyses established the molecular formula of both **1a** and **1b** as $C_{27}H_{42}O_4$ (M+ 430). The fragmentation patterns of both substances showed prominent peaks at *m/z* 139 and 115 indicative of a spiroketal moiety (2). The ir spectra showed prominent hydroxyl and carbonyl bands, as well as the characteristic bands in the fingerprint zone attributed to the spiroketal moiety. An analysis of the ir spectra indicated that **1a** was a 25*R* spirostan because the 900 cm^{-1} band was more intense than the 920 cm^{-1} band (3,4); their intensities were reversed in **1b** indicating a 25*S* configuration. The ^{13}C-nmr spectra confirmed these conclusions. The F-ring carbons of **1a** exhibited resonances at 109.1 ppm (C-22), 31.46 (C-23), 28.8 (C-24), 30.25 (C-25), 66.8 (C-26), and 16.98 (C-27). Those of **1b** showed peaks at 109.6 ppm (C-22), 26.08 (C-23), 25.8 (C-24), 27.3 (C-25), 65.2 (C-26), and 16.02 (C-27). These assignments, obtained with help of APT spectra to discriminate carbon types, agree with those of Tori *et al.* (5). With the exception of these carbons all the other signals in the ^{13}C nmr spectra of **1a** and **1b** were practically identical. The carbonyls appeared at 210.9 and the carbons carrying the hydroxyls at 69.7 ppm. A deuterated derivative (6) showed that the ketone functionality was flanked by methylenes (37.6 and 39.8 ppm). It was, therefore, inferred that the carbonyl was at C-3. This proposition was confirmed by the mass spectra of **3a** and **3b**, the ketal derivatives of **1a** and **1b**, respectively, (M+474, $C_{29}H_{46}O_5$); both substances displayed a base peak at *m/z* 99. It has been demonstrated (7) that steroids with an ethylene ketal at C-3 and no other substituent at ring A produce the most abundant fragment at *m/z* 99. If there is no substituent on ring B, the second most abundant fragment is usually at *m/z* 125. The absence of such a fragment in the mass spectra of **3a** and **3b** indicated that the hydroxyl group could be located at C-6 or C-7 in both substances.

The location of the hydroxyl group was also suggested by the chemical shifts of carbons C-5 and C-7 (53.1 and 41.8 ppm, respectively). Upon acetylation of **1a** and **1b**, these signals appeared at 50.1 and 37.6 ppm in both acetates [**1c** and **1d**]. To confirm this, **1a** was treated with chromic acid yielding a diketone with mp 233-236°, which was in good agreement with the value reported by Marker *et al.* for chlorogenone (8).

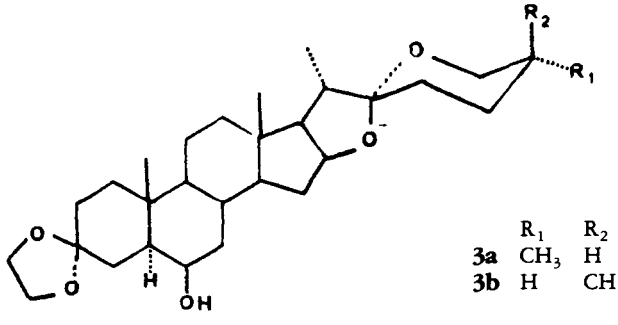
Elution of the column with EtOAc yielded a mixture of **2a** and **2b** (322 mg). The mixture was chromatographed over a small column of Si gel, but complete separation was only obtained by preparative tlc. The plates were developed 10 times with C_6H_6-EtOAc (1:1) yielding 28 mg of **2a** and 63 mg of **2b**. The first of these substances, mp 268-271°, was identified as chlorogenin by comparison of their physical properties with



	R_1	R_2	R_3
1a	CH_3	H	H
1b	H	CH_3	H
1c	CH_3	H	Ac
1d	H	CH_3	Ac



	R_1	R_2	R_3	R_4
2a	CH_3	H	H	H
2b	H	CH_3	H	H
2c	CH_3	H	Ac	Ac



	R_1	R_2
3a	CH_3	H
3b	H	CH_3

the experimental values reported by Wall *et al.* (9). The melting point and ir spectrum of **2c**, the diacetate of **2a**, were also in fair agreement with those reported in the literature (4). The catalytic hydrogenation of **1a** in EtOH rendered a product identical to **2a** (mmp, tlc, ^1H nmr, and ^{13}C nmr). It was, therefore, concluded that the hydroxyl group in **1a** was equatorial (6α-OH). Consequently, it was inferred that **1b** also had a 6α hydroxyl.

A comparison of the ir and ^{13}C-nmr spectra of **2a** and **2b** permitted the inference that **2b**, mp 262°, was the 25*S* epimer of **2a**, and it was, therefore, identified as neochlorogenin.

The possibility that **1b** would be an artifact is not likely. The epimers **1a** and **1b** were obtained from the juice in about equal proportions, which is different from the equilibrium concentrations attainable by acid catalysis (10,11). To test this assertion

and at the same time to establish the relationship between both keto-hydroxy sapogenins, pure **1b** was refluxed with HCl in EtOH solution. After 48 h most of the 25*S* compound **1b** had been converted to the more stable equatorial epimer [**1a**]. The trivial names scorpiogenin and neoscorpiogenin are proposed for these compounds.

Inasmuch as the juice was stored overnight at 6° and shaken with $CHCl_3$ the following morning, it is possible that plant enzymes could have hydrolyzed the saponins originally present. Nevertheless, the occurrence of free sapogenins is not unknown, examples having been reported by Srivastava (12). In the Solanaceae a sugar-free 3-hydroxy-4-keto sapogenin has been reported by Gonzalez *et al.* (13), and a 3-keto sapogenin has been isolated from *Solanum tovarensis* (14).

TABLE 1. Carbon-13 Chemical Shift's of (25*R*)-6-Hydroxy-5-spirostan-3-one [**1a**]; (25*S*)-6-Hydroxy-5-spirostan-3-one [**1b**]; (25*R*)-6-Acetoxy-5-spirostan-3-one [**1c**]; (25*S*)-6-Acetoxy-5-spirostan-3-one [**1d**]; Chlorogenine [**2a**]; Neochlorogenine [**2b**]; (25*R*)-6-Hydroxy-5-spirostan-3-ethylenedioxy [**3a**]; and (25*S*)-6-Hydroxy-5-spirostan-3-ethylenedioxy [**3b**]

Carbon atom	Compounds							
	1a	**1b**	**1c**[a]	**1d**[a]	**2a**	**2b**	**3a**	**3b**
C-1	38.6	38.5	38.3	38.6	37.3	37.3	36.2	36.3
C-2	37.6	37.6	37.5	37.6	29.6	29.5	31.2	31.1
C-3	210.9	210.9	210.6	210.9	71.7	71.7	109.2	109.1
C-4	39.5	39.5	39.4	39.5	30.8	30.8	32.2	32.2
C-5	53.1	53.1	50.1	50.1	51.8	51.7	50.8	50.8
C-6	69.7	69.7	72.2	72.2	69.3	69.2	69.3	69.4
C-7	41.8	41.8	37.6	37.6	41.6	41.6	42.2	42.1
C-8	34.0	34.0	33.8	33.8	33.9	34.0	34.0	34.0
C-9	53.5	53.5	53.4	53.5	53.9	53.9	53.8	53.7
C-10	36.5	36.5	36.8	36.8	36.3	36.3	36.4	36.3
C-11	21.1	21.1	21.1	21.1	21.0	21.0	21.0	21.0
C-12	39.8	39.8	39.7	39.8	39.9	39.8	39.9	39.9
C-13	40.6	40.6	40.6	40.6	40.6	40.6	40.6	40.6
C-14	55.9	55.9	55.9	55.9	56.1	56.1	56.0	56.1
C-15	31.8	31.8	31.7	31.7	31.6	31.6	31.7	31.8
C-16	80.6	80.7	80.5	80.6	80.8	80.8	80.6	80.5
C-17	62.3	62.3	62.4	62.3	62.2	62.2	62.2	62.3
C-18	16.0	16.0	16.2	16.0	15.9	15.9	16.0	16.0
C-19	12.7	12.7	12.6	12.3	13.4	13.3	12.5	12.5
C-20	41.8	42.3	41.7	41.7	42.1	42.2	42.0	42.3
C-21	14.3	14.1	14.3	14.1	14.2	14.0	14.2	14.1
C-22	109.1	109.6	109.0	109.6	109.2	109.7	109.0	109.1
C-23	31.5	26.1	31.5	26.1	31.5	26.0	31.5	26.1
C-24	28.8	25.8	28.8	25.8	28.8	25.8	28.8	25.8
C-25	30.3	27.1	30.3	27.3	30.2	27.1	30.3	27.3
C-26	66.8	65.1	66.8	65.2	66.8	65.2	66.8	65.1
C-27	16.9	16.3	16.9	16.3	16.9	16.2	17.0	16.3

[a]Acetates: **1c**, 20.8 (Me), 170.3 (carbonyl).
1d, 20.9 (Me), 170.4 (carbonyl).

This study was undertaken based on the report of Perez Medina *et al.* (1) that no glycoalkaloids were present in the fruits of *S. scorpioideum*. Such anomaly is sometimes an indication that unusual steroidal alkaloids might be present (15,16), but in this case no alkaloids were found.

EXPERIMENTAL

GENERAL.—Tlc was performed on Si gel G plates; the spots were visualized by spraying with dilute

H_2SO_4 and charring at 110°. Melting points were measured on a Fisher-Johns hot-stage and are uncorrected. Optical rotations were measured on a Zeiss polarimeter Model 53187 using a sodium lamp. The ^{1}H-nmr and ^{13}C-nmr spectra were determined in $CDCl_3$ with TMS as the internal standard in a Varian spectrometer Model FT-80A. Chemical shifts are expressed in δ values (^{1}H) and ppm (^{13}C-nmr). In addition to completely decoupled spectra, APT spectra were obtained for carbon type discrimination. The ir spectra were recorded on a Perkin-Elmer Model 377 spectrometer as KBr discs. The mass spectra were performed in a Jeol Model JMSG-2 apparatus at 70 ev using direct inlet. Microanalyses were made at Simon Bolivar University, Caracas.

PLANT MATERIAL, EXTRACTION, AND CHROMATOGRAPHY.—Green berries (7.8 kg) of *S. scorpioideum* were collected in August, 1985, in Usaquén, a suburb of Bogotä. A voucher specimen is kept at the MERF Herbarium (Herbarium of the Faculty of Pharmacy, ULA, Merida, Venezuela). The same day of collection the fruits were coarsely ground, and the juice, obtained by pressing the pulp, was shaken with $CHCl_3$. Evaporation of the solvent at reduced pressure yielded 17.2 g of extract which was treated over a Si gel column (500 g). Elution was started with C_6H_6, followed by C_6H_6/EtOAc mixtures; 500-ml fractions were taken and examined by tlc.

From fraction 26 (C_6H_6-EtOAc, 10:1) 232 mg of **1a** was obtained as crystals, mp 204-208°. Recrystalization from MeOH afforded crystals with mp 208-211°, pure on tlc, $[\alpha]_D^{21.5}-29{,}6$ (*c* 1.98, $CHCl_3$); ir ν max 3420, 1690, 985, 920, 900 cm^{-1}; ms *m/z* 430 (M^+, 21.6% $C_{27}H_{42}O_4$), 394 (11%), 358 (19.3%), 316 (34%), 298 (23%), 269 (38%), 139 (base peak, $C_9H_{15}O$), 115 (47%); ^{1}H nmr (80 MHz, $CHCl_3$) δ 0.78 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 0.94 (3H, d, *J*=7 Hz, 21-Me), 0.75 (3H, d, *J*=7 Hz, 27-Me), 2.72 (1H, dd, *J*=16 and 4 Hz), 3.35 (1H, m, C16α-H), 3.40 (1H, s, OH), 4.35 (1H, m, C6-*H*OH); ^{13}C nmr see Table 1. Found C 75.52%, H 9.61%; $C_{27}H_{42}O_4$ requires C 75.35%, H 9.77%.

Fractions 37/45 yielded 190 mg of **1b** mp 215-219°, $[\alpha]_D^{21.5}-52.7$ (*c* 4.21, $CHCl_3$); ir ν max 3420, 1690, 990, 922, 899 cm^{-1}; ms *m/z* 430 (M^+, 16%, $C_{27}H_{42}O_4$), 348 (14%), 316 (21%), 301 (10%), 298 (8%), 288 (22%), 139 (base peak, $C_9H_{15}O$) 115 (43%); ^{1}H nmr (80 MHz, $CHCl_3$) δ 0.76 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 1.05 (3H, d, *J*=7 Hz, 21-Me), 0.96 (3H, d, *J*=7 Hz, 27-Me), 2.70 (1H, dd, *J*=16 and 4 Hz), 3.32 (1H, t, *J*=12 Hz, C16-αH), 3.40 (1H, s, OH), 3.93 (1H, dd, *J*=10 and 2 Hz), 4.35 (1H, m, C6-*H*OH); ^{13}C nmr see Table 1; Found: C 75,46%, H 9.62%; $C_{27}H_{42}O_4$ requires: C 75.35%, H 9.77%. From fractions 27/36 594 mg of **1a**+**1b** was obtained. This mixture was later separated by thick layer chromatography on 1 mm thick 20×40 cm plates. Five developments with C_6H_6-EtOAc (2:1) were employed to obtain 210 mg of **1a** and 255 mg of **1b**.

Fractions 60/80 were pooled, and the residue (322 mg) was treated over a small Si gel column (50 g). Elution with C_6H_6-EtOAc (5:1) yielded a mixture of **2a** and **2b** (127 mg). Complete separation of both substances was obtained by preparative tlc using 0.5 mm 20×20 cm plates. Ten developments with C_6H_6-EtOAc (1:1) yielded 28 mg of **2a** and 63 mg of **2b**. The first of these substances [**2a**] crystallized from MeOH as fine needles, mp 268-271°, $[\alpha]_D^{21.5}-42°$ (*c* 0.40, MeOH); ir ν max 3400, 985, 922, 900 cm^{-1}; ^{1}H nmr (80 MHz, $CHCl_3$) δ 0.77 (3H, s, 18-Me), 0.84 (3H, s, 19-Me), 0.94 (3H, d, *J*=7 Hz, 21-Me), 0.78 (3H, d, *J*=7 Hz, 27-Me), 2.15 (1H, s, C3-β OH), 3.35 (1H, m, C16-αH), 3,40 (1H, s, C6-αOH), 4.35 (1H, m, C6-*H*OH); ^{13}C nmr see Table 1; Found: C 75.24%, H 10.31%; $C_{27}H_{44}O_4$ requires: C 74.96%, H 10.25%. This compound was identified as chlorogenin. Its mp and optical rotation agree with those reported in the literature (9).

The second substance [**2b**], crystallized from $(CH_3)_2CO$ mp 262°, $[\alpha]_D^{21.5}-61°$ (*c* 3.2, MeOH); ir ν max 3400, 1055, 985, 920, and 900 cm^{-1}; ^{1}H nmr (80 MHz, $CDCl_3$) δ 0.76 (3H, s, 18-Me), 0.85 (3H, s, 19-Me), 1.07 (3H, d, *J*=7 Hz, 21-Me), 0.97 (3H, d, *J*=7 Hz, 27-Me), 2.15 (1H, s, 3β-OH), 3.35 (1H, t, *J*=10 Hz, C16-αH), 3.73 (1H, s, 6α-OH), 3.92 (1H, dd, *J*=11 and 4 Hz), 4.35 (1H, m, C6-*H*OH); ^{13}C nmr see Table 1; Found: C 75.40%, H 10.12%; $C_{27}H_{44}O_4$ requires: C 74.96%, H 10.25%. A comparison of the ^{13}C-nmr spectrum of this substance with the spectrum of **2a** permitted identification as neochlorogenine.

ACETYLATION OF **1a**.—Anhydrous pyridine and Ac_2O were added to 60 mg of **1a** and left overnight at room temperature. The following morning, iced H_2O was added, and the precipitate was filtered and washed with H_2O. The acetate **1c** crystallized from Me_2CO as colorless needles, mp 164-165°; ir ν max 1740, 1708, 1250 cm^{-1}; ^{1}H nmr (80 MHz, $CDCl_3$) δ 0.75 (3H, s, 18-Me), 1.04 (3H, s, 19-Me), 0.92 (3H, d, *J*=7 Hz, 27-Me), 1.08 (3H, d, *J*=7 Hz, 21-Me), 1.99 (3H, s, acetate); ^{13}C nmr see Table 1.

ACETYLATION OF **1b**.—In the same manner as above, 60 mg of **1b** was acetylated. The acetylated derivative **1d** crystallized from Me_2CO, mp 193-195°; ir ν max 1735, 1705, 1245, 985, 920, 900 cm^{-1}; ^{1}H nmr (80 MHz, $CDCl_3$) δ 0.80 (3H, s, 18-Me), 1.10 (3H, s, 19-Me), 0.98 (3H, d, *J*=7 Hz, 27-Me), 1.08 (3H, d, *J*=7 Hz, 21-Me), 2.00 (3H, s, O-Acetate); ^{13}C nmr see Table 1.

DEUTERATION OF **1b**.—Tetrabutylammonium bromide (50 mg) and **1b** (80 mg) were dissolved in 10 ml of freshly distilled dry CH_2Cl_2. This mixture was shaken during 3 days at room temperature with 5

ml of D_2O containing 5% NaOD under dry N_2 atmosphere. The organic layer was shaken twice again with NaOD solution during 24 h. Finally the organic layer was dried over Na_2SO_4 and evaporated. The residue was purified over a small Si gel column that was eluted first with C_6H_6. Elution with a mixture of C_6H_6-EtOAc (1:1) afforded 45 mg of pure (25*S*)-6α-hydroxy[2,2,4,4-$^2H_4$]-5α-spirostan-3-one, mp 209-211°; ir ν max 3410, 1675 cm^{-1}; ^1H nmr (80 MHz, $CDCl_3$) δ 0.75 (3H, s, 18-Me), 0.99 (3H, s, 19-Me), 1.07 (3H, d, J=7 Hz, 21-Me), 0.93 (3H, d, J=7 Hz, 27-Me), 3.32 (1H, t, J=12 Hz), 3.92 (1H, dd, J=12 and 4 Hz), 4.35 (1H, m); ^{13}C nmr C-1 (38.4), C-2 (missing), C-3 (210.6), C-4 (missing), C-5 (53.0), C-6 (69.7), C-7 (41.8), C-8 (34.0), C-9 (53.6), C-10 (36.5), C-11 (21.1), C-12 (39.8), C-13 (40.6), C-14 (55.9), C-15 (31.8), C-16 (80.7), C-17 (62.3), C-18 (16.3), C-19 (12.7), C-20 (41.8), C-21 (14.1), C-22 (109.6), C-23 (26.1), C-24 (25.8), C-25 (27.1), C-26 (65.2), C-27 (16.0).

(25*S*)-3-ETHYLENEDIOXY-5α-SPIROSTAN-6α-OL [**3b**].—To obtain this ketal derivative the procedure of Herzog *et al.* (17) was followed. 50 mg of **1b** was dissolved in 25 ml of dry C_6H_6. To this solution 20 mg of P-TsOH and 0.2 ml of ethylene glycol were added, and 15 ml of C_6H_6 was distilled to eliminate H_2O by azeotropic distillation. The organic layer was washed with a solution of Na_2CO_3, dried over anhydrous Na_2SO_4, and distilled to dryness. Crystallization from MeOH yielded **3b** as fine needles, mp 253-260°, ir no carbonyl absorption; ms *m/z* 474 (M^+, 18%, $C_{29}H_{46}O_5$), 456 (3%), 402 (8%), 360 (35%), 331 (6%), 268 (8%), 251 (7%), 139 (64%), 99 (base peak, $C_5H_7O_2$); ^1H nmr (80 MHz, $CDCl_3$) δ 0.75 (3H, s, 18-Me), 0.80 (3H, s, 19-Me), 1.07 (3H, d, J=7 Hz, 21-Me), 0.92 (3H, d, J=7 Hz, 27-Me), 3.9 (4H, m); ^{13}C nmr see Table 1.

(25*R*)-3-ETHYLENEDIOXI-5α-SPIROSTAN-6α-OL [**3a**].—A solution of 50 mg of **1a** in 25 ml of dry C_6H_6 was treated in the same manner as explained above to obtain its ketal derivative [**3a**], mp 241-245°, ir no carbonyl absorption; ms *m/z* 474 (M^+, 25%, $C_{29}H_{46}O_5$), 456 (7%), 402 (10%), 360 (37%), 331 (8%), 269 (15%), 139 (51%), 99 (100%); ^1H nmr (80 MHz, $CDCl_3$) δ 0.75 (3H, s, 18-Me), 0.83 (3H, s, 19-Me), 0.94 (3H, d, J=7 Hz, 21-Me), 0.79 (3H, d, J=7 Hz, 27-Me), 3.90 (4H, m); ^{13}C nmr see Table 1.

CHROMIC ACID OXIDATION OF **1a**.—Kiliani's reagent was added drop by drop to a solution of 220 mg of **1a** in Me_2CO. After 30 min at room temperature, the reaction was complete, and a few drops of MeOH were added to destroy excess reagent. After the usual workup, the product crystallized from Me_2CO and its mp (233-236°) was found to be practically identical to the mp reported for chlorogenone (8); ir ν max 1720 and 1700 cm^{-1} (two carbonyls); ^1H nmr (80 MHz, $CDCl_3$) δ 0.96 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 1.07 (3H, d, J=7 Hz, 21-Me), 0.85 (3H, d, J=7 Hz, 27-Me), 3.55 (1H, dd, J=24 and 10 Hz), 4.2 (1H, d, J=6 Hz), 4.75 (2H, m).

CATALYTIC HYDROGENATION OF **1a**.—A solution of **1a** (50 mg) in EtOH (25 ml) was mixed with 50 mg of prereduced PtO_2, suspended in EtOH, and shaken during 4 h under an atm of H_2 at 50 psig. The catalyst was filtered off, and the solution was evaporated to dryness. The residue crystallized from MeOH, mp 268-270°, was identical to **2a** (mmp, tlc, ^1H nmr, ^{13}C nmr).

ISOMERIZATION OF **1b**.—A solution of **1b** (20 mg) in EtOH (4.5 ml) was mixed with 0.75 ml of conc. HCl and refluxed for 48 h. The reaction was followed by tlc, and it was stopped when no further change in the relative concentrations of 25*S* (Rf=0.27) and 25*R* (Rf=0.32) isomers was observed. The reaction mixture was taken to dryness and applied to a 0.5 mm thick Si gel plate (20×20 cm); the plate was developed three times with C_6H_6-EtOAc (1:1). The bands were made visible spraying H_2O and extracted with $CHCl_3$/MeOH. The upper layer yielded 9 mg of a compound identical to **1a** (tlc, mp, and ir); from the lower layer a very small amount (0.8 mg) of the original substance [**1b**] was recovered. Several by-products were visible on the tlc plate, but they were not investigated.

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LITERATURE CITED

1. L.A. Perez-Medina, E. Travecedo, and J.E. Devia, *Planta Med.*, **12**, 478 (1964).
2. H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Ellucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden Day, San Francisco, 1964, p. 110.

3. M.E. Wall, C. Roland Eddy, M.L. MacClenann, and M.E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952).
4. C. Roland Eddy, M.E. Wall, and M. Klumpp Scott, *Anal. Chem.*, **25**, 266 (1953).
5. K. Tori, S. Seo, Y. Terui, J. Nishikawa, and F. Yasuda, *Tetrahedron Lett.*, **22**, 2405 (1981).
6. C. Starks, *J. Am. Chem. Soc.*, **93**, 195 (1971).
7. G. von Mutzenbecker, Z. Pelah, D.H. Williams, H. Budzikiewicz, and C. Djerassi, *Steroids*, **2**, 475 (1963).
8. R.E. Marker, E.M. Jones, and D.L. Turner, *J. Am. Chem. Soc.*, **62**, 2537 (1940).
9. M.E. Wall, M.M. Krider, E.S. Rothman, and C. Roland Eddy, *J. Biol. Chem.*, **198**, 533 (1952).
10. R.E. Marker, A.C. Shabica, and D.L. Turner, *J. Am. Chem. Soc.*, **63**, 2274 (1941).
11. M.E. Wall, S. Serota, and L.P. Witnauer, *J. Am. Chem. Soc.*, **77**, 3086 (1955).
12. S.K. Srivastava and S.D. Srivastava, *Phytochemistry*, **18**, 1758, (1979).
13. A.G. Gonzalez, C.G. Francisco, R. Freire, R. Hernandez, J.A. Salazar, E. Suarez, A. Morales, and A. Usubillaga, *Phytochemistry*, **14**, 2483 (1975).
14. A. Morales Mendez, C. Riera, and L. Moreno, *Revista de la Facultad de Farmacia* (*ULA-Merida*), **15**, 133 (1974).
15. A. Usubillaga, C. Seelkopf, J. Karle, J. Dale, and B. Witkop, *J. Am. Chem. Soc.*, **92**, 700 (1970).
16. A. Usubillaga, A. Paredes, P. Martinod, and J. Hidalgo, *Planta Med.*, **23**, 286 (1973).
17. H.L. Herzog, M.A. Jevnik, P.L. Perlman, A. Nobile, and E.B. Hershberg, *J. Am. Chem. Soc.*, **75**, 266 (1953).

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