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ALKALOIDS FROM *SOLANUM HYPOMALACOPHYLLUM*

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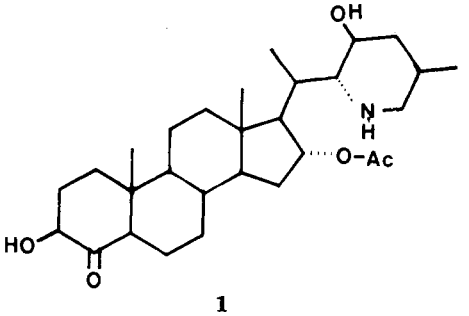
ABSTRACT.—*Solanum hypomalacophyllum* has yielded a new 4-keto steroidal alkaloid believed to be 22,26-epimino-4-oxo-5α-cholestane-3β-23β-diol (**3a**), and desacetylsolaphyllidine (**2a**) already reported in *Solanum ecuadorense*. Previous work on this plant yielded solaphyllidine (**1**) and solamaladine.

Solanum hypomalacophyllum Bitter is a small tree native to the Venezuelan Andes where it grows wild in humid places at altitudes above 2500 meters. Solaphyllidine (**1**) is the most abundant alkaloid in the green berries of this plant (1). Tlc of a $CHCl_3$ extract of the juice shows the presence of several minor alkaloids; one of them, solamaladine, was already reported (2), but its structure has been revised recently (3).

In this paper, isolation of desacetylsolaphyllidine (**2a**), an alkaloid previously found in *Solanum ecuadorense* (4), and 22,26-epimino-4-oxo-5α-cholestane-3β-23β-diol (**3a**), a new 4-keto steroidal alkaloid, is described.

DISCUSSION

The juice obtained from 30 kg of green berries was extracted with $CHCl_3$ as described in the experimental section. The crude alkaloids were dissolved in MeOH, solaphyllidine crystallized immediately upon cooling, and from the mother liquors, desacetylsolaphyllidine was obtained. Mild hydrolysis of **1** produces **2a**, and both alkaloids yield the same *N,O*-tetracetyl-derivative (**2b**).



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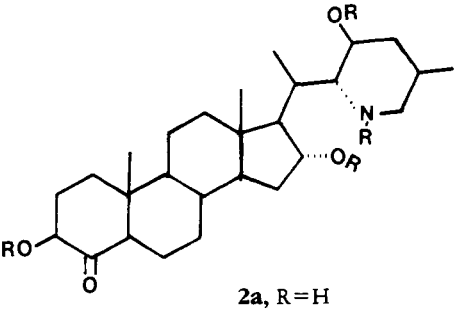
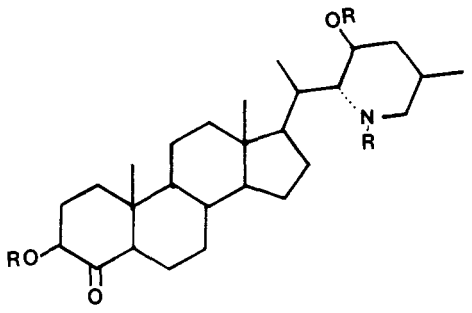


2a, R=H
2b, R=Ac

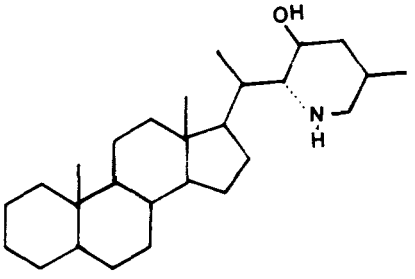
The new alkaloid (**3a**) was obtained by preparative tlc of the mother liquors of **2a**. The mass spectrum of **3a** shows a molecular ion at *m/z* 431 ($C_{27}H_{45}NO_3$), and the base peak at *m/z* 114 indicates a hydroxy methyl-piperidine side chain (5), the same as **1** and **2a**. Because one of the remaining oxygens is a carbonyl, as shown by an ir band at 1712 cm^{-1}, the other was thought to be a hydroxyl at C-3 on biogenetic grounds. The proton on C-3 appears as a triplet ($J=10$ cps) and suggests that **3a** also has a 3β-hydroxy-4-keto moiety. On Table 1, the ^{1}H-nmr (60 MHz) of **1**, **2a**, **3a**, and its acetylated derivative (**3b**) are presented and can be compared.

A mild acetylation of **3a** yields a *N,O*-triacetyl derivative (**3b**). The ^{1}H-nmr of this compound shows the presence of two *O*-acetyl and one *N*-acetyl group. The mass spectrum of **3b** shows the molecular ion at *m/z* 557 ($C_{33}H_{51}NO_6$). The most abundant fragments at *m/z* 198 (100%) and *m/z* 156 (55%) indicate the conversion of the side chain to an *N,O*-diacetyl derivative.

The existence of a 3-hydroxy-4-keto moiety in **3a** was confirmed by subjecting its acetate (**3b**) to a Huang-Minlon reduction. The carbonyl group and the 3-*O*-acetyl were removed, yielding **4** (6). The ir spectrum of **4** shows neither acetyl nor carbonyl bands. The triplet at δ 4.10 attributed to the proton on C-3 does not appear on the ^{1}H-nmr spectrum of this compound (see Table 1). This signal is always observed in 3-hydroxy-4-keto alkaloids.



3a, R=H
3b, R=Ac



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This chemical and spectroscopic evidence makes it possible to propose a 22,26-epimino-4-oxo-cholestane-3β-23β-diol structure for the new alkaloid (**3a**).

TABLE 1. ^{1}H-nmr Signals of Relevant Protons in 4-Keto Steroidal Alkaloids

Compound[a]	C-18 CH_3	C-19 CH_3	C-21 $CHCH_3$	C-27 CH-CH_3	C-22 N-C-*H*	C-23 CHR_1	C-3 CHR_1	C-16 CHR_2
1	0.69*S*	0.72*S*	0.91 d (*J*=7)	0.82 d (*J*=7)	2.95 d (*J*=10)	3.43 (sextet)	4.10 t (*J*=10)	4.96 t (*J*=6)
2a	0.69*S*	0.69*S*	0.91 d (*J*=7)	0.82 d (*J*=7)	2.87 d (*J*=10)	3.48 (sextet)	4.09 t (*J*=10)	4.17 t (*J*=6)
2b	0.70*S*	0.78*S*	0.96 d (*J*=7)	0.82 d (*J*=7)	3.35 m ($W_{1/2}$=8)	5.11 t (*J*=10)	5.26 t (*J*=10)	4.95 t (*J*=6)
3a	0.69*S*	0.70*S*	0.91 d (*J*=7)	0.83 d (*J*=7)	2.97 d (*J*=10)	3.47 (sextet)	4.09 t (*J*=10)	—
3b	0.70*S*	0.73*S*	1.07 d (*J*=7)	0.91 d (*J*=7)	3.33 m ($W_{1/2}$=8)	5.14 t (*J*=10)	5.18 t (*J*=10)	—
4	0.70*S*	0.78*S*	0.98 d (*J*=7)	0.90 d (*J*=7)	3.12 d (*J*=10)	3.60 (sextet)	—	—

[a] R_1=OH in **1**, **2a**, **3a** (In **4** only C23 OH present) R_2=OH in **2a**
R_1=CH_3COO in **2b** and **3b**. R_2=CH_3CO in **1** and **2b**.

EXPERIMENTAL

Tlc was performed on silica gel G plates using $CHCl_3$-MeOH (10:1), and the spots visualized with I_2 vapors. Melting points were determined on a Kofler hot-stage and are uncorrected. Optical rotations were measured on a Rudolph Research automatic polarimeter, model Autopol III. The ^{1}H-nmr spectra were determined in $CDCl_3$ solution with TMS as internal standard, and the chemical shifts are expressed as ^{1}H-nmr in δ values. The ir spectra were recorded on a Perkin-Elmer model 377 spectrometer as KBr disks. The mass spectra were performed at IVIC on a Hitachi Perkin-Elmer RMU-6E at 70 eV using direct inlet. Microanalyses were performed at Dr. H. Malissa, G. Reuter Laboratorium, 5251 Elbach über Engelskirchen, West Germany.

EXTRACTION AND SEPARATION.—Green berries of *S. hypomalacophyllum*, (30 kg) were collected at El Valle, a few kilometers from Mérida in May, 1979. A voucher is kept at MERF herbarium (herbarium of the Faculty of Pharmacy, University of Los Andes at Merida). The berries were crushed the same day in a hammer mill. The juice obtained was left overnight in a refrigerator for the chlorophyll to settle. The clear liquid was shaken with $CHCl_3$, and the $CHCl_3$ extract was then shaken several times with a 4% HOAc sol-

ution. The aqueous layer was made alkaline with NH_4OH and shaken with $CHCl_3$ to obtain, upon evaporation of the solvent, a mass of 10.4 g of crude alkaloids, which were dissolved in hot MeOH. Upon cooling, 3.9 g of crystals were obtained, which upon tlc showed to be mainly solaphyllidine (Rf 0.48). A second crop of solaphyllidine was later obtained (1.8 g), and the mother liquor, left to evaporate at room temperature, rendered, after a few days, a crop of impure desacetylsolaphyllidine (1.1 g).

From these crystals, **2a** was purified by repeated recrystallization from MeOH, mp 270-273°; Rf 0.22; $[\alpha]^{25}D+50°$ (c 0.20, MeOH); ir (CO) 1710 cm^{-1}; ms *m/z* 447 (M^+), 429 (M^+-18), 414, 412, 114 (base peak). Calcd for $C_{27}H_{45}NO_4.CH_3OH$; C 70.14, H 10.22, N 2.92. Found; C 69.92, H 10.18, N 2.92%.

The mother liquors of **2a** examined on tlc showed a main spot at Rf 0.35, which corresponds to **3a**, as well as **1** and **2a** and traces of minor components. This mixture was applied to ten silica gel HF plates (20 x 40 cm and 2 mm thick). After developing three times with $CHCl_3$-MeOH (10:1), the bands were visualized under uv light and scrapped off. The extract (0.47 g) obtained from the band between those of **1** and **2a** was shown on tlc to consist mainly of **3a**. It was applied to five 2-mm thick plates that were developed as previously described. The intermediate band was scraped off, and the extract was shown on tlc to consist of **3a** with traces of **1** and **2a**. The new alkaloid was purified by recrystallization from MeOH, mp 215-218°; $[\alpha]^{25}D+19.5°$ (c 0.01, MeOH); ir 3470 (OH), 3320 (NH), and 1712 (CO) cm^{-1}; ms: *m/z* 431 (M^+), 413 (M^+-18), 114 (base peak, $C_6H_{12}NO$). Calcd for $C_{27}H_{45}NO_3$; C 75.17, H 10.44, N 3.25. Found; C 74.91, H 10.48, N 3.14%.

Hydrolysis of solaphyllidine.—To a warm solution of **1** in 100 ml of MeOH, 0.5 g of K_2CO_3 in 10 ml of H_2O was added. The mixture was boiled under reflux for 3 h. A tlc test showed only one spot at Rf 0.22. Water and NH_4OH were added; upon cooling, the precipitate was filtered and washed with H_2O. The residue was crystallized from MeOH, and **2a** was obtained, identical (mp, mmp, ir, and ^1H-nmr) to the alkaloid isolated from the plant.

Acetylation of **2a**.—Anhydrous pyridine and Ac_2O were added to 0.5 g of **2a** and left overnight at room temperature. The following morning, iced H_2O was added, and the flocculent precipitate was filtered and washed with H_2O. It was dissolved in *iso*-PrOH, and crystals of **2b** with mp 204-205° were obtained; $[\alpha]^{25}D-24°$ (c 0.6, MeOH); ir 1740, 1250 (O-Ac); 1710 (CO), 1650 (N-Ac) cm^{-1}; ms: *m/z* 615 (M^+), 555 (M^+-60), 198 (base peak, $C_{10}H_{16}NO_3$), 156 (55%, $C_8H_{14}NO_2$).

Acetylation of **1**.—In the same manner as explained above, 0.2 g of **1** was acetylated, and a product exactly equal to **2b** was obtained (mp, ir, ^1H-nmr).

Acetylation of **3a**.—In the same way, 0.2 g of **3a** was acetylated with pyridine/Ac_2O. The product was crystallized from MeOH and fine needles with mp 189-192° were obtained; $[\alpha]^{25}D-23.2°$ (c 0.0056, MeOH); ir 1740, 1250 (O-Ac), 1712 (CO), and 1650 (N-Ac) cm^{-1}; ms *m/z* 557 (M^+), 497 (M^+-60), 454 (M^+-60-$COCH_3$), 198 (base peak, $C_{10}H_{16}NO_3$), 156 (55%, $C_8H_{14}NO_2$); *m/z* 123.2 (metastable peak). Calcd for $C_{33}H_{51}NO_6$; C 71.09, H 9.16, N 2.51. Found; C 71.35, H 9.05, N 2.45%.

Huang-Minlon Reduction of **3b**.—A solution of **3b** (80 mg) in diethylene glycol (4 ml) was refluxed with hydrazine hydrate (0.4 ml) for 2 h. KOH was added, and the excess H_2O and hydrazine hydrate were distilled off. The remaining solution was refluxed for another hour. After cooling, the mixture was poured on cold H_2O, and the white precipitate was filtered and washed with H_2O. The product was purified over a small alumina column (alkaline Al_2O_3, Activity II). The fraction eluted with $CHCl_3$ contained **4** (45 mg) mp 252-257°; ir, no carbonyl or acetate absorption; ms *m/z* 401 (M^+), 114 (base peak, $C_6H_{12}NO$). Calcd for $C_{27}H_{47}NO$; C 80.80, H 11.72, N 3.49. Found; C 80.53, H 11.60, N 3.32%.

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