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3β-AMINOSPIROSOLANE STEROIDAL ALKALOIDS FROM *SOLANUM TRISTE*

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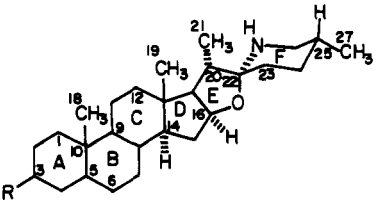
ABSTRACT.—The alkaloid fraction of the MeOH extract of the aerial parts of *Solanum triste* afforded the new steroidal alkaloid, (22*R*,25*R*)-3β-amino-5-spirosolene [**2**], and its previously synthesized dihydro derivative, (22*R*,24*R*)-3β-amino-5α-spirosolane [**3**], which is reported here for the first time as a natural product. The structures were elucidated by spectral techniques including ^{1}H-nmr, ^{13}C-nmr, and ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY nmr experiments.

The chemistry of plants of the genus *Solanum* has been extensively studied and reviewed (1,2). Steroidal alkaloids such as solasodine [**1**] and their glycosylated derivatives have been by far the most common and important compounds obtained. The extensive use of *Solanum* species in folk medicinal practice (3–6), the demonstrated clinical effects of several steroidal alkaloids (3, 7–10), and the importance of solasodine [**1**] as a starting material in the commercial production of steroidal drugs (11), have resulted in continuing studies on the genus. We are investigating some species of *Solanum* occurring in Trinidad with the aim of discovering rich sources of solasodine [**1**] and of isolating novel alkaloids with important biological activity. We report here the results of our studies on the aerial parts of *Solanum triste* Jacq. (Solanaceae), a tree of restricted distribution which has not been previously investigated.

The crude alkaloid fraction obtained from the MeOH extract of the leaves and stems of *S. triste* was subjected to vlc (12). Repeated prep. tlc ($CHCl_3$/MeOH/aqueous NH_3 on the fraction containing the major component resulted in the isolation of compounds **2** and **3**. The major compound **2** was obtained as white needles, mp 165–167°. Its ir spectrum showed an absorption band at 3300 cm^{-1}, indicating the presence of -OH and/or -NH groups. High-resolution eims gave a $[M]^+$ at *m/z* 412.3461, which corresponded to a molecular formula of $C_{27}H_{44}N_2O$, while other fragment ions were characteristic of alkaloids of the steroidal type (13). The ^{1}H-nmr spectrum (Table 1) suggested an unsaturated spirosolane-type alkaloid (2, 13–16). Thus, there were two methyl singlets (at δ 0.81 and δ 1.01) and two methyl doublets (at δ 0.94 and δ 0.84), an oxymethine proton (H-16) at δ 4.29, and signals at δ 2.60 (2H) and δ 2.65 (1H) attributable to three α-amino protons and one olefinic proton signal (δ 5.32). The ^{13}C-nmr spectrum supported the conclusions made above. It showed characteristic signals



1 R=−OH, $\Delta^{5,6}$
2 R=−NH_2, $\Delta^{5,6}$
3 R=−NH_2

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TABLE 1. Nmr Spectral Data for Compounds **2** and **3** in $CDCl_3$.

Position	Compound					
	2			**3**		
	δ_C	δ_H[a]		δ_C	δ_H[a]	
1	38.15	1.08	1.82	37.92	0.88	1.60
2	32.57	1.70	1.32	32.21	1.80	1.40
3	51.95	2.60	—	51.64	2.83	—
4	43.66	2.16	2.05	38.99	1.51	1.25
5	141.84	—		45.76	1.03	—
6	120.34	5.32		29.06	<1.16>[b]	
7	32.05[c]	1.53	2.00	32.60	0.81	1.55
8	31.56[b]	—	1.63	35.47	—	1.46
9	50.18	0.97	—	54.67	0.55	—
10	36.69	—		35.93	—	
11	20.82	1.53	1.45	21.45	1.25	1.46
12	39.98	1.16	1.74	40.60	1.10	1.71
13	40.50	—		41.10	—	
14	56.54	1.09	—	56.69	1.08	—
15	32.16[c]	1.32	2.00	32.61	1.46	2.07
16	78.73	4.29	—	78.94	4.43	—
17	62.79	1.70	—	63.81	1.78	—
18	16.41	0.81		16.98	0.89	
19	19.45	1.01		12.57	0.75	
20	41.21	—	1.88	41.78	—	1.98
21	16.27	0.94		15.83	1.08	
22	98.23	—		98.45	—	
23	34.05	<1.63>[d]		34.78	<1.72>	
24	30.30	1.42	1.60	31.22	<1.62>	
25	31.45[b]	—	1.53	31.78	—	1.62
26	47.66	2.60	2.65	48.21	2.76	2.76
27	19.29	0.84		19.90	0.82	

[a]α-Protons are on the left in the column.

[b,c]Signals with the same superscript were not well resolved in the HMQC spectrum. [While the proton assignments should be correct, carbon assignments could be interchanged.]

[d]Indicates average chemical shift for unresolved CH_2 groups.

(17) at (a) δ 98.23, indicative of the spiroaminoketal carbon (C-22); (b) δ 78.73, for the oxygenated carbon, C-16; (c) δ 47.66 and δ 51.95, assignable to C-26 and one other α-amino carbon; and (d) δ 120.34 and δ 144.84, for the carbons of an olefinic double bond. The absence of any signals around δ 3.5 in the ^{1}H-nmr spectrum and around δ 71 in the ^{13}C-nmr spectrum ruled out a hydroxy-substituted (usually 3β-substituted) spirosolane. However, the presence of an extra α-amino proton suggested that **2** was a 3-aminospirosolane steroidal alkaloid.

Confirmation of the structure of **2** as the novel (22*R*,25*R*)-3β-aminospirosolane and unambiguous assignments of the ^{1}H- and ^{13}C-nmr chemical shifts (Table 1) were achieved by using a combination of the following 2D nmr techniques: ^{1}H-^{1}H COSY (18), HMQC (19), HMBC (20), and NOESY (21). For example, the 3-amino group and the 5,6-double bond were established as follows. The H-19 methyl signal gave cross-peaks in the HMBC spectrum with the quaternary olefinic carbon signal (δ 141.84), the C-9 signal at δ 50.18 (δ 0.95), and the signal at δ 38.15 due to C-1 (δ H 1.08, 1.82). The signals of both protons on C-1 showed cross-peaks in the ^{1}H-^{1}H COSY spectrum with those of the C-2 methylene protons. The latter signals in turn showed cross-peaks with the amino-

methine proton (δ 2.60) at C-3 (δ 51.95). Cross-peaks were also observed from the H-3 signal to signals at δ 2.05 and δ 2.16 and between these H-4 signals and the olefinic proton signal at δ 5.32.

On the basis of the cross-peaks in the NOESY spectrum given by the C-18 and C-19 methyl signals and by the H-9, H-14, and H-17 signals, almost every proton in **2** could be assigned as α or β. The apparent triplet at δ 2.60 due to H-3 and one proton on C-26 had a coupling constant (J=ca. 11 Hz), which showed that both protons are axial and hence α-oriented.

Assignment of the chemical shifts of the atoms of ring F was straightforward from the data. The assigned chemical shifts for C-22 to C-26 were characteristic (17) of a (22*R*,25*R*)-spirosolane indicating that ring F is identical to that of **1**. Indeed **2** differs from **1** only in the presence of an amino instead of a hydroxy group at C-3. Their close similarity was reflected in the close correspondence of the ^{13}C-nmr chemical shifts for C-7 to C-27 of both compounds (16,22).

The more polar, minor component **3** was obtained as a pale yellow, amorphous solid with mp 177–185° [178–182° (24)]. Its ir spectrum showed an absorption at 3400 cm^{-1} (-NH and/or -OH group) and its ^{1}H- and ^{13}C-nmr spectra (Table 2) showed signals typical of a saturated spirosolane-type steroidal alkaloid (2, 13–16). As discussed for **2**, the data excluded a hydroxy substituent at C-3 and indicated an amino group instead. Comparison of the ^{13}C-nmr chemical shifts of **3** with those of the known (22*S*,25*R*)-5α,3β-aminospirosolane (soladunalinidine) (25) showed remarkably close correspondence of the chemical shifts of C-1 to C-19. The chemical shifts of C-20 to C-26 more closely matched those of the (22*R*,25*R*)-spirosolanes such as **1** and **2** (16). The ^{1}H- and ^{13}C-nmr data suggested that compound **3** was (22*R*,25*R*)-5α,3β-aminospirosolane, the C-22 epimer of soladunalinidine. The proposed structure **3** was confirmed by use of the same combination of 2D nmr methods employed for **2**. Assignment of the ^{1}H- and ^{13}C-nmr chemical shifts also proceeded along similar lines and the results are shown in Table 1. This is the first report of **3** as a natural product although it has been synthesized previously from 5,6-dihydrosolasodine (24). Further work on the isolation and structural elucidation of other minor components of *S. triste* is being conducted.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Reichert micro-melting point apparatus and are uncorrected. Uv spectra were recorded on a Perkin-Elmer 552A uv-vis spectrophotometer, and ir spectra were run as Nujol mulls using a Pye Unicam SP3-200 instrument. The ^{1}H-nmr spectra were recorded, in $CDCl_3$ using TMS as internal standard, on a Varian Unity-500 instrument. The ^{1}H-^{1}H COSY, HMQC (optimized for $^{1}J_{H\text{-}C}$=140 Hz), HMBC (optimized for $^{n}J_{H\text{-}C}$=8 Hz) and NOESY spectra were also run on this instrument, while the ^{13}C-nmr spectra (in $CDCl_3$) were run on a Varian VXR-400 spectrometer. Lreims and hreims were obtained with a Kratos/AEI MS-50 spectrometer. Si gel 60PF-254 and 366 (Merck) was used for analytical (0.25 mm) and prep. (1 mm) tlc and for vlc (11).

PLANT MATERIAL.—Aerial parts of the plant *S. triste* were collected in March 1991, near the base of the Maracas Waterfall, Maracas, St. Joseph, Trinidad. The plant material was identified by W. Johnson of the National Herbarium of Trinidad and Tobago where a voucher specimen is on deposit. The aerial parts were air dried (ca. 30°) for one week.

EXTRACTION AND ISOLATION.—The dried, milled plant material (1 kg) was exhaustively extracted by percolation with cold MeOH (10 liters). Evaporation of the solvent under reduced pressure gave a dark green gum (120 g). A portion of the gum (50 g) was dissolved in 200 ml 10% HOAc and the resulting mixture extracted with EtOAc (3×100 ml). The aqueous phase was then basified to pH 9 by addition of 2 M aqueous NH_3. The precipitate obtained was filtered off and dried to yield 3.0 g of solid. The filtrate was extracted with $CHCl_3$ (3×75 ml) followed by *n*-BuOH (3×75 ml). Evaporation of the extracting solvents yielded a further 3.5 g of extract, which was combined with the precipitate obtained earlier. Tlc analysis ($CHCl_3$-MeOH-aqueous NH_3; 85:15, saturated) of the total crude material showed one major and several minor Dragendorff-positive components. Si gel vlc (11) on a portion (3 g) of this

extract eluting with $CHCl_3$-MeOH-aqueous NH_3 mixtures of increasing polarity (9:1, saturated to 0:9:1) resulted in three combined fractions. The middle fraction (2 g) containing the major component was subjected to cc (Si gel, $CHCl_3$-MeOH-aqueous NH_3, 92:8 saturated to 80:20 saturated) to yield six combined fractions. Repeated prep. tlc ($CHCl_3$-MeOH-aqueous NH_3, 85:15 saturated) on fraction six yielded two solids (R_f 0.5 and R_f 0.35). The major, less polar, compound **2** was obtained as white needles (40 mg, 0.02%), mp 165–167° (MeOH/$CHCl_3H_2O$); $[\alpha]^{27}D$ −52° (c=0.97, MeOH); ir ν max 3250, 1550 cm^{-1}; eims *m/z* $[M]^+$ 412.3461 (17), 283.2415 (28), 138.1278 (100) $[C_9H_{16}N]^+$, 114.0925 (41) $[C_6H_{12}NO]^+$, 113.0847 (18), 56.0521 (41); ^1H- and ^{13}C-nmr data, see Table 1.

Compound **3** was obtained as an amorphous, pale yellow solid (6 mg, 0.003%); ir ν max 3400, 1600 cm^{-1}; ^1H- and ^{13}C-nmr data, see Table 2.

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