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STUDIES ON INDIAN MEDICINAL PLANTS—XLIV[1]

SOLANOFORTHINE, A NEW STEROIDAL ALKALOID FROM *SOLANUM SEAFORTHIANUM*. A NOTE ON THE MASS SPECTRUM OF SOLANOCAPSINE†

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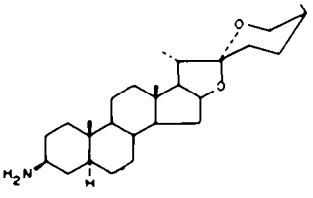
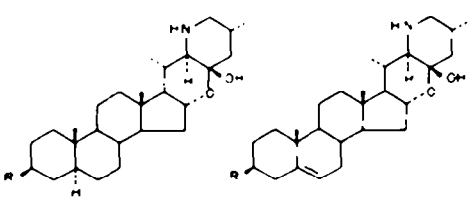
Abstract—The structure of solanoforthine, m.p. 208–10°, $[\alpha]_D$ – 26.6°, a new steroidal alkaloid isolated from *Solanum seaforthianum* Andr., has been established as 3β-amino-22,26-epimino-16α,23-epoxy-22αH,25βH-cholest-5-en-23β-ol 3, based on chemical and spectroscopic evidence and by its reduction to solanocapsine 2, another steroidal alkaloid encountered in the same species. The mass spectra of 2 and 3 are discussed.

The N.O. Solanaceae is a well-known source of steroidal alkaloids with the basic spirosolane, solanidane and 22,26-epiminocholestane skeletons.[2] It is remarkable, however, that the 3-amino function so common in *Buxus* and *Holarrhena* alkaloids[3] has rarely been encountered in *Solanum* species. In fact, jurubidine 1, 9-hydroxy jurubidine (paniculidine) and their C-25 isomers,[4] solanocapsine 2[5] and solacasine (22,N-dehydro-O-methyl solanocapsine)[6] are the only such alkaloids so far isolated from *Solanum* species. We now report a new 3-amino derivative, solanoforthine, from *Solanum seaforthianum* Andr., a plant found to have anti-hypertensive activity.[7]





2, R = NH_2 3, R = $-NH_2$
12, R = $-N{=}CHMe$ 13, R = $-N{=}CHMe$

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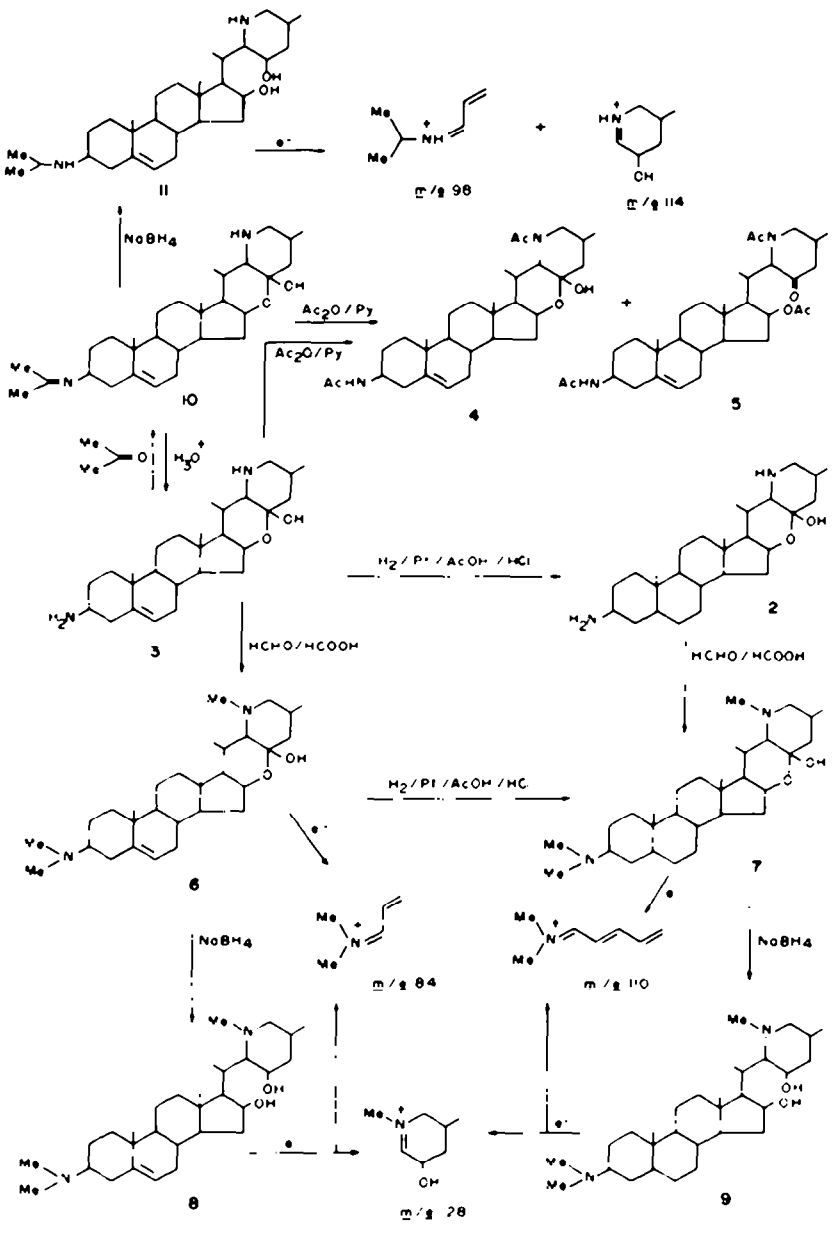
The alkaloids of *S. seaforthianum* were found to be mostly non-glycosidic in nature and were isolated without hydrolysis. The conventional procedure led to the isolation of three pairs of closely related major bases with molecular ions at *m/e* 470 and 468 (Fraction A), 456 and 454 (Fraction B) and 430 and 428 (Fraction C), the lower molecular weight components predominating in each case. Their separation proved to be extremely difficult, and only the compounds having molecular weights 468 and 428, the latter designated as solanoforthine, could be isolated pure by repeated chromatography and tedious fractional crystallisations. A GC-MS–computer study of the total alkaloids, however, revealed the presence of solasodine, solanidine and a number of 3-amino derivatives yet to be characterised.

Solanoforthine 3, $C_{27}H_{44}N_2O_2$, showed strong IR absorption for OH/NH group(s), and the presence of both was corroborated by an abundant M-18 ion and the characteristic *m/e* 56 peak of 3-amino steroids[8] in its mass spectrum. The general fragmentation pattern, however, could not be correlated with any of the known steroidal alkaloids. The NMR spectrum exhibited a signal for a vinyl proton at δ 5.31 (absent in that of its dihydro derivative) indicative of the presence of a trisubstituted double bond and a one-proton multiplet around δ 4.5 ascribed to a –CH–O– function in addition to the expected signals for C-18, C-19, C-21 and C-27 methyls of a C_{27} steroidal alkaloid.

Solanoforthine on catalytic hydrogenation yielded a dihydro-derivative 2 which could also be obtained by hydrogenation of Fraction C. Thus, the compound with MW 430 accompanying 3 in the mixture was proved to be dihydrosolanoforthine.

Acetylation of solanoforthine yielded the *N,N'*-diacetate 4 and the *O,N,N'*-triacetate 5. Both the compounds showed an abundant ion at *m/e* 494 in their mass spectra corresponding to the primary loss of a molecule of H_2O and AcOH, respectively, but the fragmentation patterns thereafter were quite distinct indicating that 5 was not the simple *O*-acetate of 4. An additional peak at



Scheme 1.

1710 cm^{-1} in the IR spectrum indicated the presence of a 6-membered ring ketone in **5**. Thus, a hemiketal linkage had to be envisaged in **3** which did not show any carbonyl absorption.

Methylation of **3** and **2** afforded the respective *N,N,N'*-trimethyl derivatives **6** and **7** which on treatment with sodium borohydride yielded the corresponding diols **8** and **9**. The mass spectra of both the diols exhibited the base peak at *m/e* 128 which must have been derived from the *N*-methyl-monohydroxy-methylpiperidine moiety generated by reduction of the hemiketal function. The mass spectra of all the compounds were consistent with a double bond at C-5 in **3** (Scheme 1).

All the available evidences thus led to a 3-amino-22,26-epiminocholestane structure for solanoforthine with a hemiketal bridge between ring D and piperidine moiety.

At this stage it became necessary to rigorously exclude a solanocapsine type structure. To our surprise, the mass spectrum of an authentic† sample of solanocapsine recorded by us proved to be quite different from the published[9] one but very close to that of solanoforthine (Fig. 1). We, therefore, isolated solanocapsine from *S. pseudocapsicum*[10] and its identity with dihydrosolanoforthine was established by comparison of the scrupulously purified compounds and their trimethyl derivatives. Since the structure and stereochemistry of solanocapsine **2** is well-established[11] that of solanoforthine **3** follows.

The first compound to be obtained pure and investigated was, however, the one with MW 468 corresponding to $C_{30}H_{48}N_2O_2$, to which structure **10** was assigned based on the following evidences: The physical data indicated the presence of OH, -CH–O, C=N and a

†Obtained through the courtesy of Prof. K. Schreiber.[10]

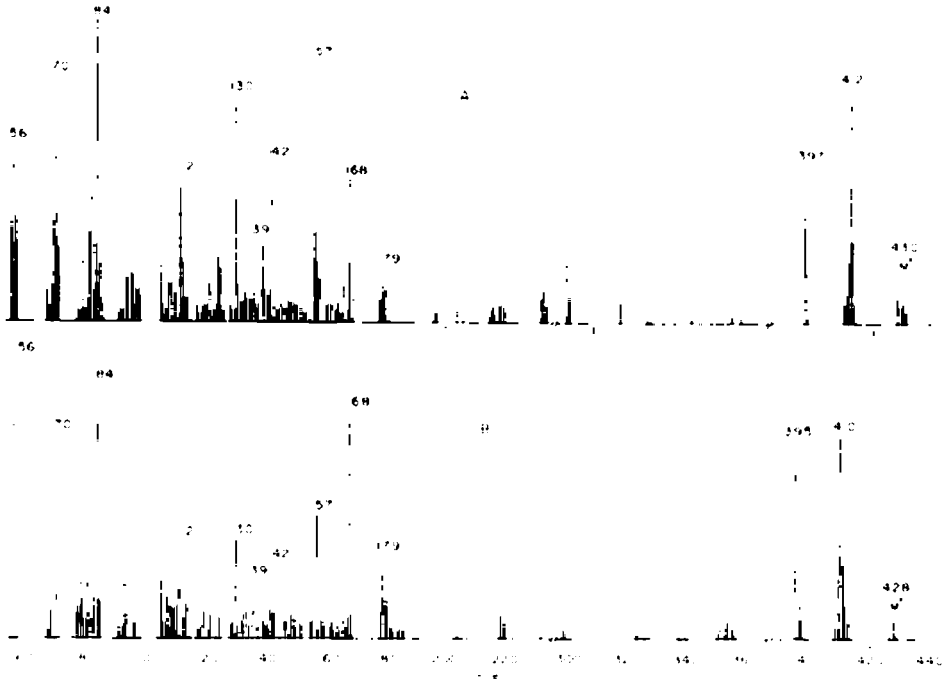


Fig. 1. Mass spectra of (A) solanocapsine (2) and (B) solanoforthine (3).

trisubstituted double bond, and the compound on acetylation yielded two acetates identical with **4** and **5** derived from **3**. Nevertheless, the respective molecular ions at *m/e* 512 and 554 were 40 mass units lower than what would normally be expected of the corresponding di- and tri-acetates of the compound. Sodium borohydride reduction of **10** furnished the tetrahydro derivative **11**. The mass spectrum of the latter showed base peak at *m/e* 114, assigned to a hydroxy-methylpiperidinium ion, indicating the presence of a hemiketal function in **10**. It also exhibited an abundant ion at *m/e* 98 corresponding to the *m/e* 56 ion of 3-amino steroids.[8] Thus, the presence of a $(CH_3)_2C{=}N$-group could be inferred in **10**, corroborated by a six proton signal at δ 2.16 in the NMR spectrum. Structure **10** was finally confirmed by its hydrolysis to **3**, which on condensation with acetone regenerated the original base.

Fraction B on hydrolysis yielded a mixture of **2** and **3**. As such, the compounds with MW 456 and 454 must be the ethylidene derivatives **12** and **13**. The structures were confirmed by the mass spectral comparison of the acetaldehyde condensation product of Fraction C.

Since acetone was used as one of the solvents for crystallisation of **10** before realization of the nature of the compounds present and compounds **12** and **13** could not be isolated by extraction of the plant materials with aldehyde free alcohol, all these compounds are considered to be artefacts.

Mass spectra of solanocapsine, solanoforthine and their derivatives

It has already been pointed out that the mass spectrum of solanocapsine recorded by us did not correspond to that published,[9] the predominant feature of which is a peak at *m/e* 114. This ion is found to be insignificant in the spectra of **2**, **3** (Fig. 1) and **10**. After recording the mass spectrum of solanocapsine under a variety of conditions we were convinced that the peak at *m/e* 114 could not have arisen from this compound. This conclusion was corroborated as follows. During the isolation of solanocapsine from *S. pseudocapsicum* it was found to be accompanied by a minor component which could be separated only with difficulty by repeated chromatography. The latter exhibited a base peak at *m/e* 114 (Fig. 2) and proved to be a mixture of two diols having gross structure **14**.† Because of the different volatility of solanocapsine and the diols, the mass spectra of their mixtures are dependent on the recording conditions. However, under appropriate conditions with approximately a 9:1 mixture of solanocapsine and the diols it was possible for us to obtain a spectrum almost identical with the published mass spectrum of solanocapsine.

The mass spectra of the three compounds **2**, **3** and **10** showed the same fragmentation pattern for the D/E/F rings with ions at *m/e* 179, 168, 157, 142, 139, 130, 112, 111, 84 and 70. The compositions of all these fragments were determined by accurate mass measurements (Table 1). Formulation of mechanistic pathways leading to these ions is difficult in the absence of labelling studies because multiple hydrogen transfers seem to be involved. What is apparent, however, is that several of the fragments could be explained by assuming initial homolysis of the C_{22}–C_{23} rather than the C_{20}–C_{22} bond of the molecular ion. Some of the fragments could be best rationalised on the basis of their genesis from the M–H_2O ion formed either by 1,2- or 1,4-elimination (Scheme 2).

Methylation of the secondary amino group led to a remarkable change of the fragmentation pattern as seen from the mass spectra (Fig. 3) of the trimethyl derivatives **6** and **7**. The conspicuous absence of peaks corresponding to the *m/e* 168 ions of **2** and **3** indicated that this peak might be derived from a M–H_2O ion formed by 1,3-elimination involving the NH proton (Scheme 2).

The trimethyl derivatives exhibit ions at *m/e* 171, 156, 126, 98 and 84 which had their counterparts in the spectra

†The structure and stereochemistry of the two diols will be discussed elsewhere

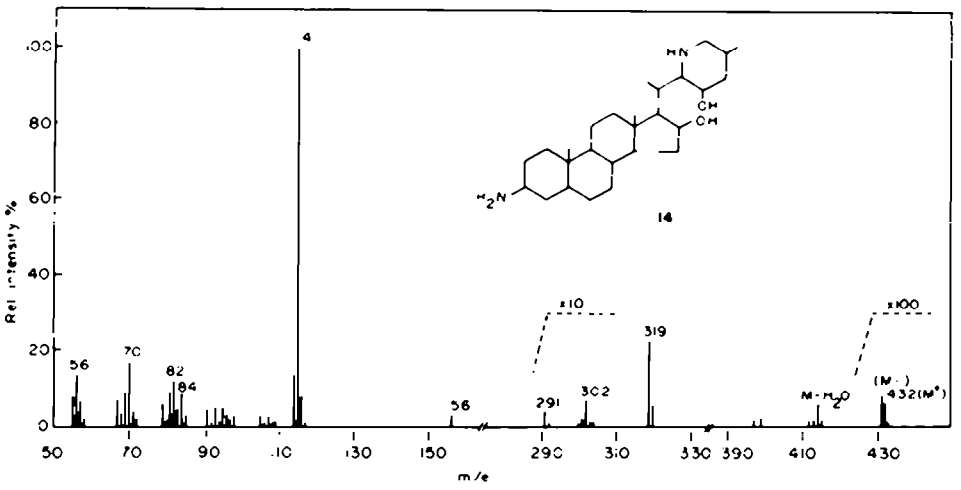


Fig. 2. Mass spectrum of the mixture of diols (14) isolated from *S. pseudocapsicum*.

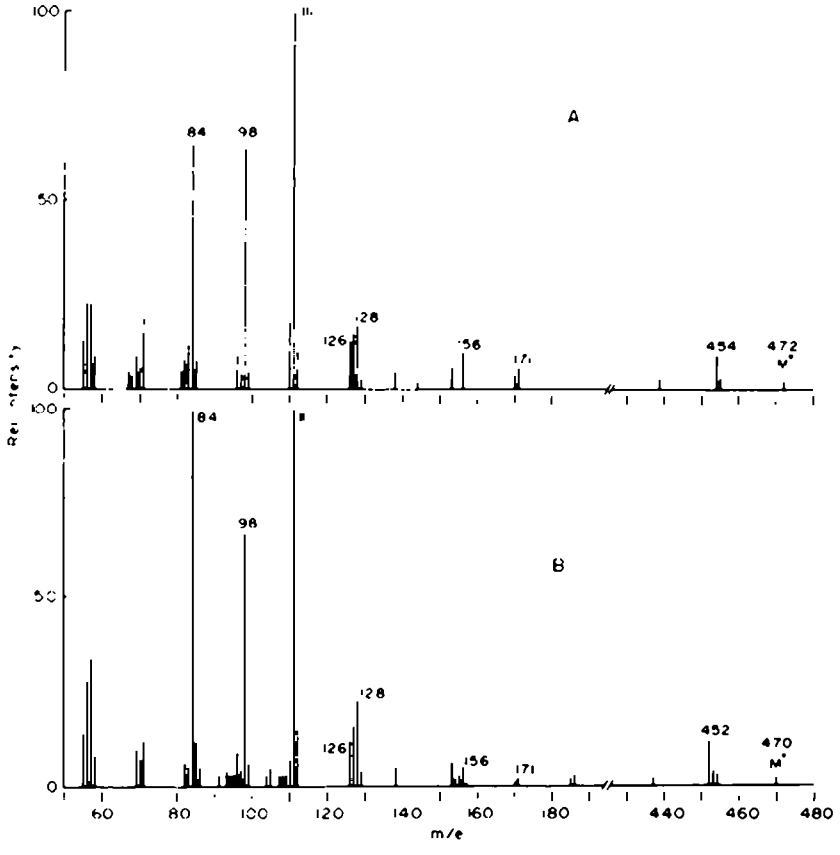
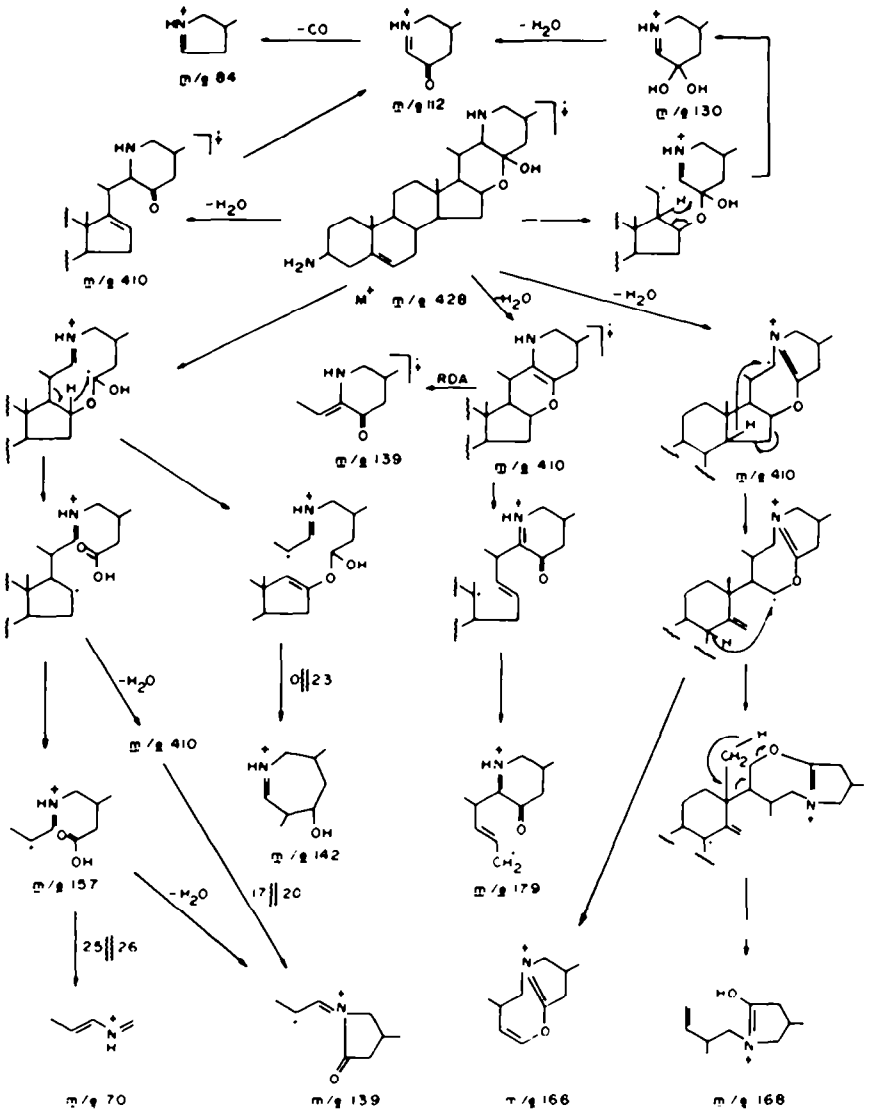


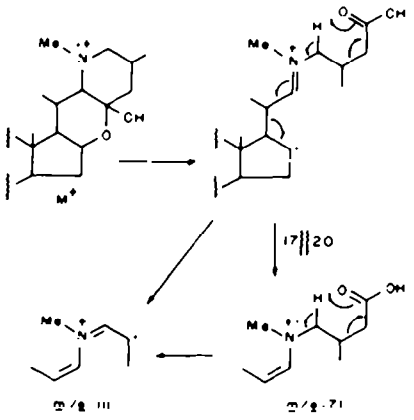
Fig. 3. Mass spectra of (A) *N,N,N'*-trimethylsolanocapsine (7) and (B) *N,N,N'*-trimethylsolanoforthine (6).

Table 1. High resolution mass spectral data for solanoforthine 3

Composition of ion	*m/e*		Composition of ion	*m/e*	
	Calc.	Found		Calc.	Found
C_4H_8N	70.0656	70.0641	$C_9H_{13}NO_2$	157.1102	157.1098
$C_5H_{10}N$	84.0813	84.0813	$C_{10}H_{16}NO$	166.1231	166.1249
$C_6H_{12}NO_2$	130.0868	130.0846	$C_{10}H_{18}NO$	168.1388	168.1407
$C_8H_{11}NO$	139.0997	139.1004	$C_{11}H_{17}NO$	179.1310	179.1337
$C_8H_{16}NO$	142.1231	142.1220			



Scheme 2.



Scheme 3.

of 2 and 3. The peak at *m/e* 128 may well be due to the homologue of the *m/e* 114 ion of solanocapsine postulated by Budzikiewicz.[9] However, the most striking feature in these spectra are the base peaks at *m/e* 111, because there were no corresponding peaks in the spectra of the unmethylated compounds. The most probable pathway of the genesis of this ion is shown in Scheme 3.

EXPERIMENTAL

All m.ps were determined in open capillaries in a sulphuric acid bath and are uncorrected. IR spectra were recorded as Nujol mulls in an Infracord-137 spectrophotometer. Optical rotations are in $CHCl_3$, measured in a Hilger-Watts M-511 photoelectric polarimeter. NMR spectra were recorded in $CDCl_3$ with TMS as internal standard. Low resolution mass spectra were recorded with Hitachi RMU-6L instrument (electron energy 80 eV, direct inlet temp 150–190°; source temp 180–220°, source pressure $\sim 1.0 \times 10^{-6}$ Torr). High resolution mass spectra were recorded with a CEC 21-110B instrument.[12] TLC's were done on silica gel (Gouri Chemicals, Calcutta) plates with the solvent system C_6H_6:EtOAc (2:8) saturated with ammonia.

Isolation of the alkaloids

Dried and powdered stem-bark (3 kg) of *Solanum seaforthianum* Andr. was extracted with alcohol in a Soxhlet apparatus for 30 h, the extract concentrated (150 ml) under reduced pressure and poured into 2N acetic acid (1 l) and extracted with chloroform

(3 × 200 ml). The acid solution was treated with a saturated NaCl solution and centrifuged. The separated crude hydrochloride was suspended in water, made basic with ammonia, and extracted with chloroform to yield 16 g of total alkaloids.

The total alkaloid was dissolved in hot benzene (40 ml) and allowed to stand at room temperature. The separated coloured solid (1.0 g) on crystallisation from chloroform–acetone afforded "Fraction A" (0.7 g) consisting of two compounds of MW 468 and 470 (MS).

The residue from the mother liquor was dissolved in chloroform (100 ml) and extracted with acetate buffer (pH 4.6). The extact was treated with an excess of oxalic acid, the separated oxalate salts were filtered, and the free bases (5.2 g) regenerated. Attempted redissolution of this material in chloroform left an insoluble solid (50 mg) which was found to be a mixture of two components (TLC) with MW 428 and 430 (MS) and was designated as "Fraction B". The chloroform-soluble part was chromatographed on alumina. Elution with 50% ether in petroleum ether (4 l) afforded a crystalline solid (3.0 g) which on repeated crystallisation from methanol afforded "Fraction C" (1.2 g) consisting of two compounds having MW 454 and 456 (MS). The mother liquors on evaporation and mass spectral study revealed the presence of four components of MW 428, 430, 454 and 456. This material could not be resolved further and was hydrolysed (*vide infra*) with 2N-ethanolic HCl to yield a sample identical with "mixture B". Attempted separation of "Fraction C" also proved futile and on similar hydrolysis afforded "Fraction B".†

Isolation of **10**. "Fraction A" on repeated chromatography over silica gel afforded a viscous oil (0.2 g) which on crystallisation from chloroform–acetone furnished fine needles of **10** (0.1 g), m.p. 209–211°; $[\alpha]_D$ – 37.7° (*c* 0.53); ν_{max} 3200–3000 (NH/OH), 1660 (C=N) cm^{-1}; δ(100 MHz) 0.78 (18-Me), 1.0 (19-Me), 2.16 (Me_2C=N–), 4.50 m (16-H), 5.3 m (6-H); *m/e* (rel. intensity) 468 (M^+, 12), 450 (76), 435 (60), 393 (10), 340 (13), 339 (14), 312 (8), 272 (17), 179 (45), 168 (100), 166 (33), 157 (56), 142 (28), 139 (33), 130 (28), 112 (56), 96 (85), 84 (59), 70 (74). (Found: MW 468.3754. $C_{30}H_{48}N_2O_2$ requires: 468.3715).

The compound with MW 470 was, however, not obtained pure.

Isolation of solanoforthine **3**. "Fraction B" (1.0 g) on repeated crystallisation from chloroform-petroleum ether and then from benzene yielded **3** (0.15 g), m.p. 208–210°; $[\alpha]_D$ – 26.6°, (*c* 0.6); ν_{max} 3420–3100 (NH_2, NH, OH), 1605–1595 (NH_2) cm^{-1}; δ(90 MHz) 0.79 (18-Me), 1.00 (19-Me), 0.87 d and 0.92 d (21-Me and 27-Me), 4.50 m (16-H), 5.31 m (6-H). (Found: MW 428.3411. $C_{27}H_{44}N_2O_2$ requires: 428.3402). The compound of MW 430 **2** could not be isolated pure.

Preparation of **2**. A solution of "Fraction B" (0.1 g) in glacial acetic acid (10 ml) was hydrogenated over PtO_2 (75 mg) in presence of a few drops of conc. HCl. After 4 h fresh catalyst (75 mg) was added and the reaction allowed to proceed for 4 h more. The reaction product after work up contained a single component (TLC) which on crystallisation from chloroform-petroleum ether afforded **2** (60 mg), m.p. 199–201°; $[\alpha]_D$ + 10.7° (*c* 0.56); ν_{max} 3500–3100 (NH_2, NH, OH), 1600 (NH_2) cm^{-1}; identical (IR, TLC, MS) with solanocapsine isolated (*vide infra*) from *S. pseudocapsicum*. (Found: MW 430.3576. $C_{27}H_{46}N_2O_2$ requires: 430.3559). Hydrogenation of **3** under identical conditions also yielded **2**.

Acetylation of **3**. A solution of **3** (60 mg) in pyridine (2 ml) was treated with Ac_2O (1 ml) at room temp. for 12 h and the product chromatographed over alumina. Elution with 25% chloroform in benzene (200 ml) yielded a solid (25 mg) which on crystallisation from acetone afforded the *O,N,N'*-triacetate **5** (12 mg), m.p. 226–227°; ν_{max} 1730 and 1235 (OAc), 1710 (C=O), 1640 and 1630 (two NAc) cm^{-1}; *m/e* (rel. intensity) 554 (M^+, 0.1), 511 (2), 494 (26), 452 (5), 435 (57), 420 (4), 402 (6), 392 (6), 342 (14), 340 (11), 312 (10), 281 (45), 253 (50), 181 (20), 179 (18), 155 (88), 126 (28), 112 (96), 84 (42), 60 (79), 43 (100).

†The TLC behaviour of the Fractions A, B and C were identical, each showing two spots corresponding to **2** and **3**. Apparently the ethylidene and isopropylidene derivatives were hydrolysed on TLC plates.

Further elution with 5% methanol in chloroform afforded an oil which on rechromatography and crystallisation from acetone yielded the *N,N'*-diacetate **4** (9 mg), m.p. 220–221°, ν_{max} 1640 and 1630 (two NAc) cm^{-1}; *m/e* (rel. intensity) 512 (M^+, 0.5), 494 (12), 479 (10), 451 (4), 435 (10), 420 (5), 380 (100), 321 (12), 296 (11), 281 (9), 253 (10), 221 (44), 166 (50), 162 (57), 156 (80), 139 (50), 126 (74), 114 (54), 112 (45), 84 (90), 60 (49), 43 (68).

Methylation of **3**. Compound **3** (60 mg) was dissolved in 98% formic acid (0.5 ml), 36% formaldehyde solution (1 ml) was added and mixture was heated on a steam bath for 2 h. The product on chromatography over alumina and crystallisation from chloroform-petroleum ether furnished the *N,N,N'*-trimethyl derivative **6** (40 mg), m.p. 201–203°, $[\alpha]_D$ + 5.3° (*c* 1.08); ν_{max} 3100–2600 (OH) cm^{-1}; δ(60 MHz) 0.81 (18-Me), 0.98 (19-Me), 2.27 (3-NMe_2), 2.43 (26-NMe), 4.5 m (16-H), 5.31 m (6-H).

Methylation of **2**. Methylation of **2** (80 mg), under the same conditions as mentioned above yielded the *N,N,N'*-trimethyl derivative **7** (60 mg), m.p. 221–223°; $[\alpha]_D$ + 42.6° (*c* 1.22); ν_{max} 3200–2600 (OH) cm^{-1}; δ(60 MHz) 0.78 (18-Me and 19-Me), 2.27 (3-NMe_2), 2.43 (26-NMe), 4.47 m (16-H) identical (m.p., m.m.p, TLC, IR, MS) with *N,N,N'*-trimethylsolanocapsine.

Hydrogenation of **6** *to* **7**. The trimethyl derivative **6** (60 mg) was hydrogenated over PtO_2 in acetic acid solution containing a few drops of HCl to yield the 5,6-dihydro derivative **7** (40 mg), m.p. 221–223°, identical with *N,N,N'*-trimethylsolanocapsine.

Sodium borohydride reduction of **6**. Compound **6** (20 mg) was reduced with sodium borohydride (40 mg) in ethanol (5 ml) at room temp. for 12 h. Usual work-up and crystallisation from chloroform-petroleum ether afforded **8** (8 mg), m.p. 236–238°; ν_{max} 3300 and 3175 (OH) cm^{-1}; *m/e* (rel. intensity) 472 (M^+, 0.3), 471 (M-1, 0.4), 454 (0.6), 328 (1), 186 (3), 185 (3), 170 (2), 169 (3), 155 (6), 128 (100), 98 (8), 84 (55).

Sodium borohydride reduction of **7**. Trimethylsolanocapsine **7** (50 mg) was reduced with sodium borohydride (100 mg) in EtOH–THF (1:1) mixture (10 ml) at room temp. for 12 h. Usual work-up and purification through PLC and crystallisation from chloroform-petroleum ether yielded **9** (20 mg), m.p. 225–226°; $\nu_{max}^{CHCl_3}$ 3200–3100 (OH) cm^{-1}; *m/e* (rel. intensity) 474 (M^+, 1), 473 (M-1, 3), 456 (2), 330 (6), 186 (2), 185 (2), 170 (8), 155 (4), 128 (100), 110 (28), 98 (12), 84 (42).

Sodium borohydride reduction of **10**. A solution of **10** (2 mg) in methanol (1 ml) was treated with $NaBH_4$ (10 mg) at 0° and then kept overnight at room temp. Usual work-up gave a product characterised as **11** from its mass spectrum, *m/e* (rel. intensity) 472 (M^+, 0.6), 471 (1), 457 (0.6), 454 (0.7), 375 (14), 359 (25), 342 (8), 289 (5), 274 (4), 214 (3), 180 (5), 114 (100), 98 (100), 84 (12), 70 (15), 56 (32).

Hydrolysis of **10**. Compound **10** (20 mg) was dissolved in 2N HCl in 50% aqueous ethanol (10 ml) and kept at room temp. for 24 h. After removal of the solvent under reduced pressure, the residue was dissolved in hot water and filtered. The filtrate was made basic with NH_4 and extracted with chloroform. The oily product on crystallisation from chloroform-petroleum ether afforded **3** (10 mg), m.p. 208–210°.

Acetylation of **10**. Acetylation of **10** with Ac_2O/pyridine at room temp. followed by chromatography of the products yielded two compounds identical with **4** and **5**.

Isolation of solanocapsine from S. pseudocapsicum. Dried and powdered leaf and stem-bark (1 kg) was extracted by cold percolation with 2N-acetic acid (5 l) for 72 h. The extract was filtered, washed with $CHCl_3$, made basic with ammonia and extracted with chloroform. The organic phase was washed, dried, and distilled. The oily residue (1.0 g) was chromatographed over alumina. Elution with 40% chloroform in petroleum ether (1 l) yielded solanocapsine, which crystallised from chloroform-petroleum ether as colourless flakes (0.7 g), m.p. 208° and is identical with **2** (IR, TLC, MS).

A sample of this material was methylated (HCHO/HCOOH) as described earlier to yield the trimethyl derivative identical (IR, m.p., m.m.p., TLC, MS) with **7**.

The later chromatographic fractions on rechromatography yielded a mixture of two diols which could not be resolved by TLC. However, the *N,N,N'*-trimethyl derivatives of these diols could be separated. The spectral data of the diols and their

trimethyl derivatives indicated both the diols to have the gross structure **14**.

Preparation of **10**. A solution of **3** (50 mg) in EtOH (5 ml) and acetone (5 ml) was refluxed for 3 h. Distillation of the solvent and crystallisation of the residue yielded **10** in quantitative yield.

Preparation of **12** *and* **13**. Fraction C (10 mg) in EtOH (5 ml) was refluxed for 3 h in the presence of a few drops of acetaldehyde. The mass spectrum of the product was identical to that of Fraction B indicating it to be mixture of **12** and **13**.

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