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SPERMINE ALKALOIDS FROM *SCHWEINFURTHIA PAPILIONACEA*

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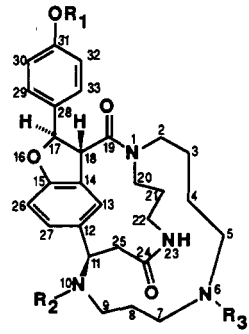
ABSTRACT.—Two new macrocyclic alkaloids, 11-*epi*-ephedradine A (11-*epi*-orantine) [**1**] and schweinine [**2**], were isolated from the whole plant of *Schweinfurthia papilionacea*, in addition to (−)-ephedradine A (orantine) [**3**]. Their structures were determined by spectroscopic means, and the stereochemistry has been assigned on the basis of 2D nmr techniques.

Schweinfurthia papilionacea (Burm.f.) Boiss. [syn. *Schweinfurthia sphaerocarpa* (Benth.) A. Braun., *Antirrhinum papilionaceum* Burm.f., *Antirrhinum glaucum* Stock., *Linaria sphaerocarpa* Benth.] (Scrophulariaceae) is locally known as "sannipat" and is found in Karachi (Sind) and Lasbella (Baluchistan) (1,2). This drug is considered to act as a tonic, promote diuresis, and reduce fever in typhoid conditions (3). The powdered herb is snuffed in cases of nose bleeding. The leaves are said to be used in the treatment of diabetes (4). No work on the chemical constituents of this plant is reported so far. In the present communication we report the isolation and structure determination of three alkaloids, 11-*epi*-ephedradine A (11-*epi*-orantine) [**1**], schweinine [**2**], and (−)-ephedradine A (orantine) [**3**]. Alkaloid **3** has previously been isolated from *Ephedra* roots (5). Compounds **1** and **2** are new alkaloids.

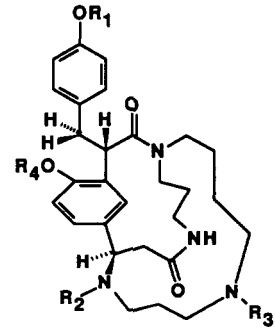
RESULTS AND DISCUSSION

The whole plant of *S. papilionacea* was extracted with cold EtOH, and the solid obtained was partitioned between H_2O and EtOAc. The aqueous portion was basified to pH 9 with NH_3, and the crude alkaloids were extracted with $CHCl_3$. The crude alkaloidal mixture was subjected to cc and preparative tlc, leading to the isolation of two new macrocyclic spermine alkaloids, 11-*epi*-ephedradine A (11-*epi*-orantine) [**1**] and schweinine [**2**], as well as (−)-ephedradine A [**3**]. Compound **3** was identified as (−)-ephedradine A (orantine) by extensive application of one- and two-dimensional high field nmr techniques and through comparison of its spectral data with those reported in the literature (5,6).

The molecular formula of **1** was determined as $C_{28}H_{36}N_4O_4$ by hrms indicating thirteen double bond equivalents in the molecule. The mol wt of **1** was confirmed by fabms, fdms, and cims. Analysis of the uv, ir, ^{1}H-nmr, ^{13}C-nmr, and ms data of **1**



1 $R_1=R_2=R_3=H$, H-11α
3 $R_1=R_2=R_3=H$, H-11β
5 $R_1=R_2=R_3=Ac$, H-11α



2 $R_1=R_2=R_3=H$, $R_4=Ac$
4 $R_1=R_2=R_3=R_4=Ac$

suggested a structure closely related to (−)-ephedradine A (orantine) [3]. Acetylation of **1** under normal conditions afforded one *N,N,O*-triacetate **5**. A double doublet at δ 3.95 (J = 1.23, 5.5 Hz) in **1** was assigned to H-11. The smaller coupling constant as compared to that of H-11 in **3** (δ 4.09, dd, J = 13 and 8 Hz) showed that the dihedral angle between H-11 and H-25 is nearly 60° in **1**. 2D nmr measurements were carried out to verify the ^{1}H-nmr assignments. The coupling interactions and spatial proximities were established through correlated spectroscopy (COSY-45) and NOESY. The relationship between carbons and their respective protons was identified by hetero-COSY experiments. The relative stereochemistry at various centers was deduced from the results of nOe difference experiments. Irradiation at δ 4.66 (H-18β) caused no nOe at δ 6.05 (H-17α), suggesting the trans stereochemistry for these protons. At the same time, a strong nOe effect was observed for H-29 and H-33, which confirmed the β orientation for the aromatic ring at C-17.

Irradiation of H-11 produced a significant nOe at H-13 (9.7%) in addition to the expected strong nOe effect (11%) at δ 3.7 (H-25α). No nOe was observed between H-11 and H-27. These nOe interactions served to establish that in the preferred conformation the H-11 proton lies close to the H-13 and therefore has an α configuration, as also indicated by the Dreiding model. Additional proof for the absolute configuration was provided by cd spectroscopy. The negative Cotton effects at 226 (Δε −13.3) and 283 (−2.17) nm were correlated with the 17*R*,18*R* configuration for these chiral centers. Comparison of the cd curve of **1** with that of **3** revealed some discrepancies in the pattern and rotatory strengths at 222, 234, and 283 nm, which were due to the alteration of the dipole coupling between the transition moment of the dihydrobenzofuran and phenyl moieties, as a consequence of the *R* configuration at C-11. Thus, the structure of **1** represents the C-11 epimer of the known (−)-ephedradine A [3].

The structure of **2** was assigned on the basis of spectral studies and comparison with the closely related compounds, seco-aphelandrine (7) and seco-orantine (6). Compound **2** showed a characteristic uv spectrum with λ max at 226, 276, and 305 (sh) nm indicating the presence of a *p*-substituted phenol moiety attached to the dihydrobenzofuran grouping. The absorption at 226 nm showed a red shift of 14 nm on addition of 0.1 N methanolic NaOH, indicating the presence of a phenolic hydroxyl group which was also confirmed by a positive $FeCl_3$ test. When the ir spectrum of **2** was compared to that of **1**, it showed an intense peak at 1740 cm^{-1}, demonstrating the presence of an ester group. The parent peak indicated by hrms at *m/z* 536.297930 was appropriate for the molecular formula $C_{30}H_{40}N_4O_5$. Compound **2** gave a triacetate **4**, showing that it had three groups that can be acetylated. These are two secondary amino and one phenolic hydroxyl group as in **1**. In the ^{1}H-nmr spectrum for **2**, no H-17 methine proton signal was observed; an H-17 methine proton signal was present in **1** at δ 6.05 (d, J = 11.25 Hz). A methylene signal was present at δ 3.3 (dd, J = 4.12 Hz) and 2.84 (dd, J = 4, 6 Hz). Furthermore the ^{1}H-nmr signals for H-26 and H-27 at δ 6.55 and 5.95 were not consistent with the corresponding signals for **1** (δ 6.75 and 7.15). The change in the chemical shift of H-26 and H-27 was presumably due to the proximity of the acetate group. Compound **2** also showed a singlet at δ 2.05, which integrated as three protons and was attributed to the methyl of the acetate group. The close correspondence of the other signals with those of **1**, the absence of the H-17 methine proton, and the presence of two additional double doublets for the H-17 methylene indicated that C-17 is attached to the phenyl but not to the ether oxygen and the bond between O-16 and C-17 was not present. Compound **2** was therefore considered to be an acetyl derivative of 16,17-seco-ephedradine with an acetoxyl substitutent attached at C-15.

In order to study the structural modification in **2** and to verify the assignments in the ^{1}H-nmr spectrum, a comprehensive series of homodecoupling experiments and 2D

nmr measurements including heterocosy, COSY-45, NOESY and *J*-resolved nmr measurements were carried out. Although these studies were unsuccessful due to the flexible nature of the compound, the relative stereochemistry was finally decided by nOe difference measurements. The configurations of H-11 and H-18 were found to be the same as in **1**. Irradiation at δ 6.55 (H-26) resulted in 15.5% nOe at δ 2.1 ($OCOCH_3$) and 8.63% nOe at δ 5.95 (H-27), suggesting the location of the acetate at C-15 and its close proximity to H-26. Placement of the acetate group at C-15 is consistent with the ^{1}H-nmr spectrum, but more conclusively this location resulted in the anticipated shifts in the ^{13}C-nmr spectrum of **2** relative to that of **1**. The differences in the chemical shifts of C-15, C-26, and C-27 ($\Delta\delta = -7$, $+24$, and -2.35 ppm, respectively) determined the position of the acetate group unambiguously as C-15. Furthermore, it also showed the expected differences in the chemical shifts of C-17, C-18, and C-28 ($\Delta\delta = -39.71$, -2.97, and $+2.11$, respectively) on transformation of the C-17 methine carbon attached to the ether oxygen in **1** to the benzylic methylene carbon in **2** (Table 1).

The efforts to prepare a suitable crystal for X-ray crystallography were unsuccessful. Compound **2** has been given the trivial name schweinine, and its structure corresponded to the *O*(16)-acetyl-16,17-dihydro-16,17-seco-derivative of 11-*epi*-ephedradine A [**1**], which is rare in the plant kingdom.

Ephedradine A was evaluated for mutagenic activity (8) against the $his^- \mapsto his^+$ TA 98 and TA 100 tester strains of *Salmonella typhimurium*, and it was found that this alkaloid causes reversion of the tester strains by frame shift and base pair substitutions (9). The TA 98 strain had shown 22-fold increases, while TA 100 showed approximately 4-fold increases. This alkaloid caused reverse mutation after microsomal activation, thus showing that it may be a procarcinogen that is converted to a potential carcinogen after being processed by the S9 mix (10,11).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Gallenkamp and Büchi 535 apparatus and were not corrected. Optical rotations were measured with a Schmidt & Haensch polartronic-D polarimeter. Cd was obtained on a JASCO-600 CD spectropolarimeter. Uv spectra were recorded on Shimadzu UV240 and Hitachi U-3200 spectrophotometers using MeOH as solvent. Ir spectra (KBr) were taken on a JASCO A-302 spectrometer. The mass spectra were measured on Varian MAT-112 S and MAT-312 double focusing mass spectrometers connected to an MAT-188 data system and a PDP 11/312 computer system. Eims were recorded on a Finnigan MAT-112S (Varian 188 data system with PDP 11/34 DEC computer system) mass spectrometer. Cims was taken using a Finnigan MAT-112S mass spectrometer and isobutane as the reagent gas. Hreims was carried out by peak matching and by computerized measurements on a DEC PDP 11/34 computer linked to a Finnigan MAT-312 mass spectrometer. Fdms, fims, and fabms measurements were performed on an MAT-312 mass spectrometer. Fabms spectra were obtained in the positive mode using glycerol as matrix and argon as the reagent gas. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM-300 spectrometer in CD_3OD using TMS (δ = 0) as internal standard (δ units).

PLANT MATERIAL.—The plant *S. papilionacea* was collected in November 1985, from the Karachi University Campus. A voucher specimen (No. 21573 KUH) is lodged in the Herbarium, Department of Botany, University of Karachi.

EXTRACTION AND FRACTIONATION.—Freshly collected whole plant of *S. papilionacea* (40 kg) was chopped into small pieces and finely homogenized with an Ultra-turrax homogenizer. It was then exhaustively extracted with 95% EtOH at room temperature. The residue obtained on evaporation of the EtOH extract was partitioned between EtOAc and H_2O. The aqueous layer was basified with NH_3 (pH 9.0) and extracted repeatedly with $CHCl_3$. The alkaloid-containing $CHCl_3$ layers were combined, washed, dried, and concentrated to afford a crude alkaloidal mixture (47 g). The crude alkaloidal mass was subjected to cc over Si gel (1.5 kg, 60–230 mesh, E. Merck) using as eluents $CHCl_3$ and $CHCl_3$/MeOH mixtures of increasing polarity.

ISOLATION AND CHARACTERIZATION OF 11-*epi*-EPHEDRADINE A (11-*epi*-ORANTINE) [**1**].—The fraction (260 mg) obtained from the cc (Si gel type 60) with $CHCl_3$-MeOH (7.5:2.5) showed the pres-

TABLE 1. ^{13}C-nmr Spectral Data of Compounds **1–5**.

Carbon	Compound				
	1[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]
2	49.29	50.66/50.94[c]	47.23	50.72/50.91[c]	51.03
3	27.01	27.62/28.98[c]	27.68	28.63/28.88[c]	27.96/28.22[c]
4	28.95	26.82/27.0[c]	27.06	26.17/26.91[c]	29.52/29.73[c]
5	47.63	48.63/48.86[c]	46.01	49.05/49.33[c]	46.62/46.83
7	47.63	47.42/47.71[c]	46.00	47.8/48.0[c]	45.89
8	25.62	25.6/26.01[c]	25.65	25.02/25.55[c]	26.08/26.22[c]
9	47.63	46.18/46.40[c]	45.00	46.41/46.8[c]	45.32
11	61.85	59.94	60.11	57.18	57.15
12	136.41	142.96	134.96	133.29	131.10
13	130.62	128.95	128.72	123.81/124.22[c]	124.34
14	131.27	128.45	127.16	129.52/129.82[c]	125.14
15	160.50	153.26	158.4	150.62	150.3
17	89.61	49.9	88.3	47.49	86.79/87.01[c]
18	54.47	51.5	52.97	49.98	54.29
19	173.72	172.11/172.19[c]	172.00	170.03	171.51
20	44.16	44.14/44.50[c]	43.81	44.4/45.1[c]	44.34/46.67[c]
21	24.03	24.25	24.03	24.28/24.58[c]	25.5
22	41.86	41.7/41.86[c]	41.91	41.05/41.7[c]	39.59
24	171.86	173.36/172.87[c]	171.2	172.07	170.46
25	38.69	38.37/38.58[c]	36.48	37.98/38.21[c]	37.54
26	110.08	134.51/134.6[c]	108.33	131.75/132.16[c]	110.5
27	123.84	121.49	121.84	121.49/121.61	132.96
28	127.49	129.6	126.54	136.53	138.22
29	128.91	131.25/131.36[c]	127.10	127.25/127.54[c]	127.33
30	116.33	116.49/116.91[c]	115.27	121.68/121.9[c]	121.91
31	159.02	159.06/159.26[c]	157.8	150.1	150.64
32	116.33	116.49/116.91[c]	115.27	121.68/121.9[c]	121.91
33	128.91	131.25/131.36[c]	127.10	127.25/127.54[c]	127.33
OCOMe		174.0/174.2[c]		171.04	
				171.64	172.14[d]
$OCOCH_3$		21.50/21.71[c]		21.52	21.05
				22.41	
NCOMe				168.14	169.75[d]
				169.07/169.29[c]	169.65[d]
$NCOCH_3$				20.99/21.14[c]	21.74
				21.79/21.95[c]	22.52

[a]Recorded at 75 MHz in CD_3OD in ppm.
[b]Recorded at 75 MHz in $CDCl_3$ in ppm.
[c]The doubling of signals is due to the presence of interconverting conformers.
[d]Assignments may be interchanged.

ence of a major alkaloid on tlc, which was purified by preparative tlc on Si gel plates using $CHCl_3$-MeOH (8:2), whereby about 100 mg of a pure alkaloid, named 11-*epi*-ephedradine A (11-*epi*-orantine) [**1**], was obtained: mp >200° (dec); $[\alpha]_D$ −13 (c = 0.03, MeOH); uv (MeOH) λ max 230, 277 (sh), 280, 291 (sh) nm; (MeOH/MeONa) λ max 246, 274, 290 nm; ir (KBr) ν max 3420, 3270 (NH, OH), 3050, 2950, 2860, 1631 (CONH), 1520, 1490, 1450, 1360, 1270, 1250, 1220, 1170, 1110, 960, 840 cm^{-1}; ^{1}H nmr (300 MHz, CD_3OD) δ 7.8 (s, NH-23), 7.28 (d, J = 8.5, H-29, H-33), 7.2 (s, H-13), 7.15 (d, J = 8.0, H-27), 6.79 (d, J = 8.6 Hz, H-30, H-32), 6.75 (d, J = 7.9 Hz, H-26), 6.05 (d, J = 11.25 Hz, H-17), 4.66 (d, J = 11.25, H-18), 3.95 (dd, J = 1.25, 5.5, H-11α), 3.7 (m, H-25α), 2.6 (m, H-25β), 1.7–4.0 (m, methylene protons); ^{13}C nmr see Table 1; eims m/z (rel. int. %) $[M+1]^+$ 493 (15), $[M]^+$ 492 (26), 449 (42), 420 (4), 406 (10), 377 (15), 351 (6), 322 (5), 278 (8), 266 (6), 265 (9), 252 (10), 251 (15), 250 (10), 242 (5), 238 (20), 236 (21), 224 (7), 223 (10), 214 (9), 210 (9), 209 (9), 198 (8), 184 (11), 181 (7), 169 (30), 165 (8), 155 (26), 153 (13), 141 (3), 129 (15), 121 (21), 113 (19), 110 (22), 107 (25), 100

(23), 98 (100), 94, 84, 70, 56; hreims *m/z* $[M]^+$ 492.27103 (calcd for $C_{28}H_{36}N_4O_4$, 492.27363), $[M-C_2H_5N]^+$ 449.22987 (calcd for $C_{26}H_{31}N_3O_4$, 449.23144), $[M-(C_2H_5N_2)_2]^+$ 406.18897 (calcd for $C_{24}H_{26}N_2O_4$, 406.18924), 380.17289 (calcd for $C_{22}H_{24}N_2O_4$, 380.17359), 377.18385 (calcd for $C_{23}H_{25}N_2O_3$, 377.18650), 351.1672 (calcd for $C_{21}H_{23}N_2O_3$, 351.17085), 322.1413 (calcd for $C_{18}H_{18}N_4O_2$, 322.142967), 265.08638 (calcd for $C_{17}H_{13}O_3$, 265.08646), 238.09122 (calcd for $C_{15}H_{12}NO_2$, 238.08679), 223.07585 (calcd for $C_{15}H_{11}O_2$, 223.07589), 209.09537 (calcd for $C_{13}H_{11}N_3$, 209.09529), 169.13348 (calcd for $C_9H_{17}N_2O$, 169.13403); cd (EtOH, $c=66.6\times10^{-5}$) λ max (Δε) 223 (−10.5), 226 (−13.3), 231 (−9.1), 234 (−6.5), 255 (−0.69), 283 (−2.17), 295 (0), 297 (−0.55).

ACETYLATION OF COMPOUND **1**.—Compound **1** (25 mg) was acetylated in the same manner as compound **2** to yield *N*(6),*N*(10),*O*(34)-triacetyl-11-*epi*-ephedradine A [**5**], which exhibited the following data: mp 178–180°; $[\alpha]_D$ −10.1 ($c=0.069$, MeOH); uv (MeOH) λ max 212, 282, 287 nm; ir (KBr) ν max 3350, 3320 (br NH), 1764 (ester carbonyl), 1636 (CONH), 1548, 1499 cm^{-1}; ^1H nmr (300 MHz, CD_3OD) δ 7.87 (s, NH-23), 7.55 (br s, H-13), 7.43 (d, $J=8.5$ Hz, H-29, H-33), 7.21 (d, $J=8.17$ Hz, H-30, H-32), 7.17 (d, $J=8.16$), 6.92 (dd, $J=3.5$, 8.2 Hz, H-27), 6.78 (d, $J=8.5$ Hz, H-26), 6.42 (br d, $J=9.09$ Hz, H-17), 5.54 (br t, H-11), 4.64 (d, $J=9.12$ Hz, H-18), 2.27 (s, OAc), 2.22, 2.10, 2.06 (3s, 6H, NAc); ^{13}C nmr (75 MHz, $CDCl_3$) see Table 1; eims *m/z* (rel. int. %) $[M]^+$ 618 (1), 575 (3), 533 (1), 327 (2), 311 (3), 309 (2), 306 (13), 269 (10), 268 (3), 265 (18), 264 (100), 256 (4), 236 (42), 210 (8), 207 (11), 169 (8), 155 (11), 149 (11), 129 (12), 113 (17), 109 (11), 100 (28), 98 (16), 95 (13), 84 (25), 81 (18); hrms *m/z* $[M]^+$ 618.3015 (calcd for $C_{34}H_{42}N_4O_7$, 618.30533), $[M-2(OAc)]^+$ 534.27707 (calcd for $C_{30}H_{38}N_4O_5$, 534.2842), 306.088016 (calcd for $C_{17}H_{12}N_3O_3$, 306.087860), 264.078680 (calcd for $C_{17}H_{12}O_3$, 264.07864), 236.08256 (calcd for $C_{16}H_{12}O_2$, 236.08372), 100.07601 (calcd for $C_5H_{10}NO$, 100.076234).

ISOLATION AND CHARACTERIZATION OF SCHWEININE [**2**].—The fractions collected from the Si gel 60 (70–230 mesh) column with $CHCl_3$-MeOH (8:2) as the solvent system were further subjected to flash cc on Si gel (230–400 mesh) by gradient elution with *n*-hexane, *n*-hexane/$CHCl_3$, and $CHCl_3$/MeOH. Fractions eluted with $CHCl_3$-MeOH (8:2) were finally purified by preparative tlc on Si gel in $CHCl_3$-MeOH-NH_3 (9:1:0.1) to afford pure amorphous alkaloid schweinine [**2**]: mp 263° (dec); $[\alpha]_D$ +61.7 ($c=0.648$, MeOH); uv (MeOH) λ max 202, 226, 276, 305 (sh) nm; (MeOH/MeONa) λ max 207, 240, 302, 330 (sh) nm; ir ($CHCl_3$) ν max 3400, 3271 (br NH, OH), 2950, 2850, 1740 (C=O), 1640 (CONH), 1520, 1470, 1380, 1220, 1170, 1070, 840 cm^{-1}; ^1H nmr (300 MHz, CD_3OD, 80° C) δ 7.30 (d, $J=8.2$ Hz, H-29, H-33), 7.1 (br s, H-13), 6.75 (d, $J=8.5$ Hz, H-30, H-32), 6.55 (m, H-26), 5.95 (m, H-27), 4.23 (dd, $J=13$, 6 Hz, H-18), 3.99 (dd, $J=5.1$, 1.3 Hz, H-11), 3.30 (dd, $J=12$, 4 Hz, H-17), 3.20 (m, H-5), 3.15 (m, H-7), 2.84 (dd, $J=4$, 6 Hz, H-17), 2.05 (s, $OCOCH_3$), 1.4–4.0 (m, methylene protons); ^{13}C nmr see Table 1; fabms *m/z* $[M+H]^+$ 537; hrms *m/z* $[M]^+$ 536.297930 (calcd for $C_{30}H_{40}N_4O_5$, 536.2998495); cd (EtOH, $c=4\times10^{-6}$) λ max (Δε) 214 (−16.0), 221 (+9.8), 224 (−4.46), 230 (+17.7), 243 (−3.65), 270 (+43.8), 288 (+23.4), 296 (+6.4), 308 (+0.2).

ACETYLATION OF COMPOUND **2**.—Compound **2** (50 mg) was acetylated in Ac_2O/pyridine (5 ml/0.5 ml) at room temperature overnight. Workup in the usual manner afforded *N*(6),*N*(10),*O*(34)-triacetylschweinine [**4**]: ir ($CHCl_3$) ν max 3440 (NH), 1760 (C=O), 1665, 1630 (CONH), 1511 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ 7.4 (d, $J=8.5$, H_2-29, H_2-33), 6.57–7.2 (5H, aromatic protons), 5.06 (d, $J=5.2$, 1.6 Hz, H-11), 4.27 (br dd, $J=13$, 6.2 Hz, H-18), 4.16 (dd, $J=13$, 6 Hz, H-17), 2.34, 2.29, and 2.27 (each s, 6H, $OCOCH_3$), 2.09, 2.03, 1.96, and 1.89 (each s, 6H, $NCOCH_3$), 1.4–4.0 (m, methylene protons); ^{13}C nmr see Table 1; eims *m/z* (rel. int. %) $[M]^+$ 662 (1), 620 (3), 577 (5), 535 (2), 396 (2), 331 (10), 313 (2), 271 (6), 268 (4), 256 (5), 242 (3), 218 (7), 210 (5), 198 (4), 190 (6), 187 (4), 169 (42), 147 (30), 129 (9), 121 (26), 107 (33), 100 (33), 84 (50), 70 (60), 60 (100); hrms *m/z* $[M]^+$ 662.33595 (calcd for $C_{36}H_{46}N_4O_8$, 662.3315396).

ISOLATION AND CHARACTERIZATION OF (−)-EPHEDRADINE A (ORANTINE) [**3**].—Fractions eluted with $CHCl_3$-MeOH (7:3) showed a major alkaloid on tlc. It was purified by repeated cc on Si gel using Me_2CO-MeOH (8.7:1.3) to afford 150 mg of the pure alkaloid: mp 170° (dec) [lit. (5) 166° (dec)]; $[\alpha]^{20}_D$ ($c=0.20$, MeOH); ^{13}C nmr (75 MHz, CD_3OD) see Table 1. All the spectral properties were identical to those previously described for (−)-ephedradine A [**3**] (6).

MUTAGENICITY ASSAY.—The *Salmonella*/Ames test, commonly named Mutatest (8), is used to detect mutagenic chemicals or metabolites capable of inducing base-pair substitution and frame-shift, point mutations. The Ames test is based on histidine$^-$↦histidine$^+$ reversion, i.e., his$^-$ auxotrophy to his$^+$ prototrophy (9).

The Ames *Sa. typhimurium*/microsomal fraction was standardized (12), and the alkaloid, ephedradine A, was screened for its mutagenic/carcinogenic potential. The spontaneous reversion rate (his$^-$↦his$^+$) of

the tester strain was measured with and without mammalian (rat) liver microsomal (S9) fraction (9×10^3 g supernatant fraction) (13) as a routine mutagenicity experiment and is expressed as the number of spontaneous revertants per plate. In a uniform background lawn of auxotrophic bacteria, the revertant colonies were clearly visible. The spontaneous reversions of a particular tester strain have a characteristic frequency. The spontaneous mutation control was run in triplicate for each strain to check the variation in results. The induced reversion mutation with and without S9 activation by ephedradine A is presented in Table 2.

TABLE 2. Reversion Mutability ($his^- \mapsto his^+$) Potential of Ephedradine A and a Control Mutagen in the *Salmonella*/Microsome Test.[a]

Mutagens	TA97a[b]		TA98		TA100	
	$S9^-$	$S9^+$	$S9^-$	$S9^+$	$S9^-$	$S9^+$
Spontaneous reversion	144	153	31	36	114	187
Nitrosoguanidine (10 μg/plate)	—	—	—	—	[c]	[c]
Ephedradine A (500 μg/plate)	0	α	81	778	143	461

[a]Spontaneous reversion (per plate) values were subtracted. Each value is the average of at least 3 experiments.

[b]Tester strains of *Salmonella typhimurium*.

[c]Uncountable.

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