249. Piperaceae Alkaloids: Part IV[1]) Structure and Synthesis of Cyclostachine A, Cyclostachine B and Cyclopiperstachine[2])

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Dedicated to Professor *T. R. Govindachari* on the occasion of his 60th birthday

Summary. Three new alkaloids designated as cyclostachine A (**2**), cyclostachine B (**7**) and cyclopiperstachine (**10**) have been isolated from *Piper trichostachyon C. DC.* Their structures have been derived on the basis of spectral and degradative studies and confirmed by synthesis. The ^{1}H- and ^{13}C-NMR. spectra of these compounds are discussed.

The isolation and structure elucidation of the alkaloid piperstachine (**1**) (from *Piper trichostachyon C.DC.*) were reported recently [3]. This structure was confirmed subsequently by synthesis [1]. Besides piperstachine, we have isolated three closely related alkaloids – cyclostachine A (**2**), cyclostachine B (**7**) and cyclopiperstachine (**10**). All three alkaloids are racemic as shown by CD. and ORD. determinations. We report here the structure elucidation and synthesis of these alkaloids. A preliminary communication on the structure of cyclostachine A has already been published [4]. The alkaloids, isolated by cold percolation of the plant with hexane, were purified by chromatography.

Cyclostachine A (**2**). – Cyclostachine A, m.p. 136–138°, $C_{22}H_{27}NO_3$ (M^+: *m/e* 353), has in the IR. spectrum ν_{max}^{KBr} 1630 cm^{-1} (tertiary amide). Its ^{1}H-NMR. spectrum showed the presence of a methylenedioxyphenyl group, an isolated disubstituted double bond and an acylpyrrolidine moiety. The ^{1}H-NMR. spectral assignments are presented in Table 1. The ^{13}C-NMR. spectrum of cyclostachine A showed the absence of quaternary carbon atoms in the aliphatic region. (For the ^{13}C-NMR. data see Table 2 and discussion on page 2300). The UV. spectrum, λ_{max}^{EtOH} 235, 287 nm (log *e* 3.65, 3.62), showed the absence of conjugation of the double bond with the aromatic ring. Its mass spectrum exhibited the molecular ion peak at *m/e* 353 and prominent ions at *m/e* 255, 218, 135 and 98 due to the ions (a) – (d), the ions (b) and (c) arising by rearrangement.

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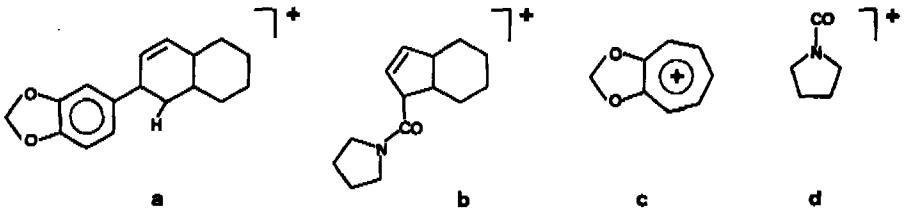
[1]) For Part III, see [1].

[2]) Contribution No. 410 from *Ciba-Geigy Research Centre*; ^{13}C-NMR. Spectroscopy, Part 13. For Part 12 see [2].

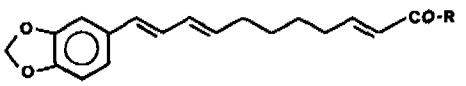
Acid hydrolysis of cyclostachine A gave pyrrolidine identified by GC. and MS. [5]. Catalytic reduction afforded the dihydro derivative **3**. The presence of a disubstituted double bond was confirmed by OsO_4-Oxidation to the diol **4** which on cleavage with periodate gave the dialdehyde **5**. The ^{1}H-NMR. spectrum of the latter showed the presence of two aldehyde protons – one as a doublet at 9.67 ppm (J =

Scheme 1



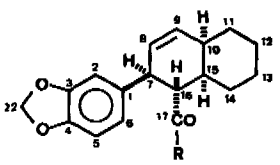
a **b** **c** **d**

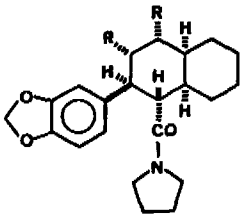
Scheme 2

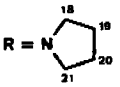


1 R = $NHCH_2CH(CH_3)_2$

9 R =  **11** R = OCH_3

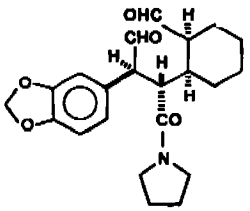




2 R = 

10 R = $NHCH_2CH(CH_3)_2$ **3** R = H

12 R = OCH_3, **14** R = OH **4** R = OH



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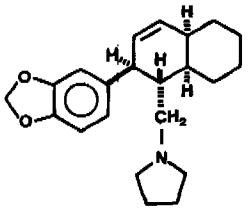

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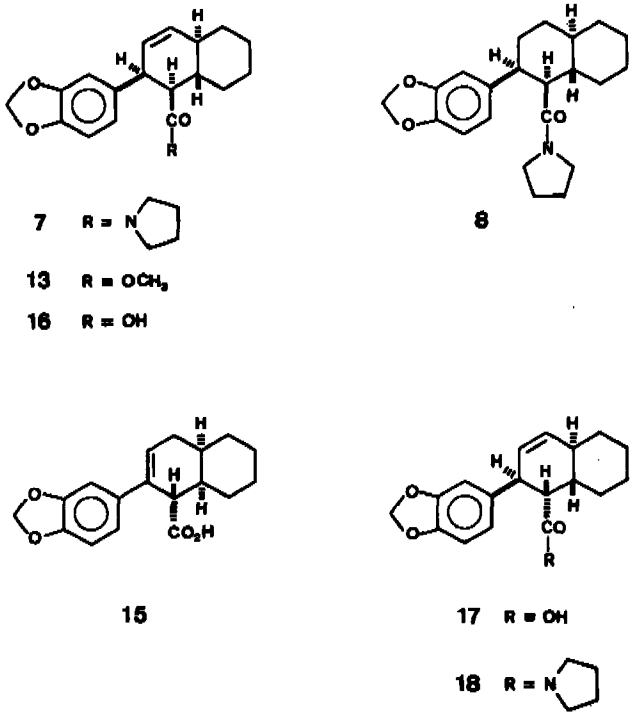
Scheme 3



2 Hz) and the other as an apparent triplet at 9.74 ppm ($\bar{J} = 1.5$ Hz) wherein the additional splitting is probably due to long-range coupling. Reduction of cyclostachine A with lithium aluminium hydride gave the amine **6** isolated as its sulfate, m.p. 110°, the structure of which was determined by X-ray crystal analysis [4]. Fig. 1 depicting molecule **6** as found in the crystal shows that atoms C(7), C(8), C(9), C(10) and C(15) in the cyclohexene ring are coplanar and C(1), C(14) and C(17) are all axially disposed. This is in contrast to ^{1}H-NMR. results which show that the amide **2** in solution has these three substituent atoms equatorial (see formula below). The nearly *trans*-diaxial arrangement of H(15), H(16) and H(7) is in good agreement with the observed coup-

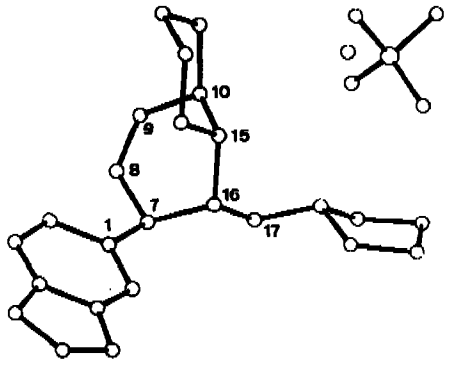
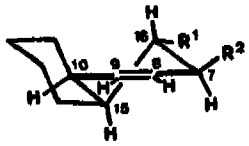


Fig. 1. *Stereoscopic drawing of the structure of* **6**-*hydrogensulfate* [4]

ling constants ($J_{15,16} = 11.0$ Hz, $J_{7,16} = 10.0$ Hz). The pseudo-axial position of H(7) is confirmed by the rather large allylic coupling (2.5 Hz) with H(9). The H(9)–C(9)–C(8) plane and the projection of the C(7)–H(7) bond form an angle of ~80°, and maximum allylic coupling $^4J^{\pi}_{H,H}$ is expected for 90°. Also the 5 Hz coupling of H(9)

Scheme 4



2 R^1 = CO–N⟨ ⟩ $R^2 = C_6H_3O_2CH_2$

10 R^1 = CO–NHCH$_2$CH(CH$_3$)$_2$

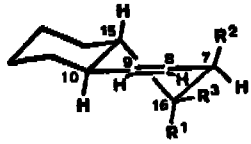
$R^2 = C_6H_3O_2CH_2$

12 R^1 = COOCH$_3$ $R^2 = C_6H_3O_2CH_2$

with H(10) is consistent with a torsional angle of ~30° (measured in *Dreiding* model). The experimentally observed large vicinal coupling constants between H(7), H(16) and H(15) cannot be explained on the basis of the conformation shown in Fig. 1 or by boat conformations of the cyclohexene ring. The conformation of the amine in the solid state is different probably because of the greater volume required by the non-planar amine ring compared with the amide form and the need to proximate the sulfate ion to the nitrogen atom for the ionic and hydrogen-bonded linkage.

Cyclostachine B (**7**). – Cyclostachine B, m.p. 135–136°, $C_{22}H_{27}NO_3$, is isomeric with cyclostachine A and has similar UV., IR. and mass spectral behaviour (see Exper. Part) indicating it to be a stereoisomer of cyclostachine A. The assignments in its ^{1}H-NMR. spectrum are presented in Table 1, the ^{13}C-NMR. data in Table 2. Catalytic reduction gave the dihydroderivative **8**. The aryl-octahydronaphthalene skeleton of cyclostachine A (**2**) is clearly originating from a triene-amide **9** related to piperstachine (**1**). Intramolecular *Diels-Alder* reaction of **9** would be expected to give the two racemates **2** and **7**. This suggested that cyclostachine B could have the

Scheme 5



7 R^1 = H R^3 = CO–N⟨ ⟩

$R^2 = C_6H_3O_2CH_2$

13 R^1 = H R^3 = COOCH$_3$ $R^2 = C_6H_3O_2CH_2$

18 R^1 = CO–N⟨ ⟩ R^3 = H

$R^2 = C_6H_3O_2CH_2$

trans-stereochemistry depicted in formula **7**. Its ^{1}H-NMR. spectrum is in excellent agreement with the conformation shown below. H(16) appears as a double doublet with $J_{16,15} = 10.5$ Hz and $J_{16,7} = 6.0$ Hz demonstrating an ax,ax arrangement of H(15) and H(16) and a pseudo-equatorial position of H(7). The axial position of H(16) is further supported by the chemical shift (2.80 ppm) which is very nearly the same as in cyclostachine A (2.76 ppm). The $J_{9,10}$ coupling is very small and not resolved in agreement with the torsional angle of $\sim 80°$ in the above conformation. The splitting patterns of the two olefinic protons are compatible with the above structure, *i.e.* only slight broadening for the H(9) doublet and extensive splitting of the H(8) doublet due to vicinal coupling with H(7) and allylic coupling with H(10). Thus, the splitting patterns of H(8) and H(9) are just opposite to cyclostachine A, in agreement with the opposite conformation of the cyclohexene ring.

Cyclopiperstachine (10). – Cyclopiperstachine, m.p. 220°, $C_{22}H_{29}NO_3$, is isomeric with piperstachine (**1**) and has spectral properties (see Exper. Part) similar to cyclostachines A and B. Its MS. and ^{1}H-NMR. spectra show the presence of an isobutylamide group in place of the pyrrolidine moiety present in cyclostachines A and B. The mass spectrum exhibits the molecular ion peak at *m/e* 355 and prominent ions **a** and **c** at *m/e* 255 and 135. The ^{1}H-NMR. spectral assignments are shown in Table 1. These data suggest structure **10** for the alkaloid, the conformation shown above (as for **2**) best explaining the H,H coupling constants between H(8), H(9), H(7) and H(10).

Synthesis. – Intramolecular *Diels-Alder* reaction of the ester **11** [1] gave a mixture of the two esters **12** and **13** which were separated by chromatography and crystallisation. The ^{1}H-NMR. spectrum of the major product supports its formulation as **12** with the conformation shown above for **2**, in agreement with expectations [6] [7]. The ^{1}H-NMR. spectrum of the minor isomer shows it to have the other possible structure **13** with the conformation given for **7**.

Alkaline hydrolysis of ester **12** yielded the acid **14** which was converted to the amide **2**, identical in all respects with naturally occurring cyclostachine A. Esterification of the acid **14** with diazomethane gave back the ester **12**, thus proving the absence of any isomerisation during the alkaline hydrolysis. When vigorous conditions were employed in the hydrolysis, isomerisation took place to yield the acid **15**, whose UV. spectrum showed the presence of a methylenedioxystyrene chromophore. Its ^{1}H-NMR. spectrum showed the presence of only one vinylic proton at 6.07 ppm (*t*, $J = 3$ Hz). Treatment of the acid chloride of **14** with isobutylamine gave the amide **10**, identical in all respects with natural cyclopiperstachine. Amide **10** was also obtained by thermal cyclisation of piperstachine (**1**).

Alkaline hydrolysis of the ester **13** gave the acid **16** which was converted to the amide **7**, identical in all respects with natural cyclostachine B. Vigorous conditions during the hydrolysis of **13** resulted in epimerisation at C(16) and yielded the acid **17**. Whereas H(16) in acid **16** appears at 2.75 ppm as a double doublet, in acid **17** it occurs as a broad singlet at 2.6 ppm. Acid **17** was converted to the amide **18** which is an isomer of cyclostachines A and B. Its ^{1}H-NMR. spectrum is reported in Table 1 and agrees well with the conformation depicted for **7**. In this compound, H(16) appears as a doublet ($J = 3.5$ Hz) at 2.72 ppm. Double resonance shows that the

Table 1. *^{1}H-NMR. data*[a])

Compound	H(2,5,6)	H(7)	H(8)	H(9)	H(10)
Cyclostachine A **2**	6.7 m	3.68 d,q $J_{7,16}$ 10.0	5.55 br d $J_{8,9}$ 10	5.9 d,d,d $J_{9,8}$ 10 $J_{9,10}$ 5 $J_{9,7}$ 2.5	3.15 d,t $J_{10,11a}$ 10 $J_{10,11e}$ 6 $J_{10,9}$ 6
Cyclostachine B **7**	6.4-6.7 m	3.0-4.0	5.55 m	5.7 d $J_{9,8}$ 10	3.0-4.0
Amide **18**	6.74 m	3.3-3.7	5.6 d,d,d $J_{8,9}$ 10 $J_{8,7}$ 4 $J_{8,10}$ 2	5.8 d,t $J_{9,8}$ 10 $J_{9,10}$ 1.5 $J_{9,7}$ 1.5	3.3-3.7
Cyclopiperstachine **10**	6.64 m	3.66 m	5.5 br d $J_{8,9}$ 10	5.8 d,d,d $J_{9,8}$ 10 $J_{9,10}$ 5 $J_{9,7}$ 3	2.0-m
Ester **12**	6.5-6.8 m	3.65 d,q $J_{7,16}$ 10	5.5 br d $J_{8,9}$ 10	5.8 d,d,d $J_{9,8}$ 10 $J_{9,10}$ 4.5 $J_{9,7}$ 2.5	2.0-m
Ester **13**	6.4-6.8 m	3.5-3.8 m	5.4-5.9 m		

[a]) All spectra were taken in $CDCl_3$ at 100 MHz except for compound **13** which was run at 60 MHz. Chemical shifts are in δ [ppm], s singlet, d doublet, t triplet, q quartet, m multiplet, br broad; J values are in Hz.

doublet splitting is due to coupling with H(15) which is at $\sim$1.6 ppm, but not with H(7). Since the two substituents at C(16) and C(7) are both axial a minor distortion of the cyclohexene half chair to release steric 1,3-diaxial interactions with H(10) and H(15) respectively, would make the torsional angle for the H(7) and H(16) protons $\sim$90° so that no coupling should be observed for these protons, as it is found in the spectrum.

The ^{13}C-NMR. spectral data (Table 2) fully support the structures of cyclostachine A (**2**), cyclostachine B (**7**) and of the synthetic stereoisomer **18**. The spectra exhibit 22 lines in the case of **2** and 21 lines with one aliphatic CH_2 signal of double

H(15)	H(16)	H(18)	H(21)	H(22)	Misc.
2.0-2.5 m	2.76 d,d $J_{16,15}$ 11.0 $J_{16,7}$ 10.0	3.4 t J 6.5	2.0-2.5 m	5.90 s	
1.0-2.2	2.80 d,d $J_{16,15}$ 10.5 $J_{16,7}$ 6.0			5.88 s	
1.6 m	2.72 d $J_{16,15}$ 3.5			5.92 s	
2.4	2.3 m	2.9 t $J_{18,19}$ 7 $J_{18,NH}$ 7	0.68 0.72 d $J_{20,19}$ 7 $J_{21,19}$ 7 (CH-$\underline{Me}_2$)	5.86 s	5.25 br t (NH) $J_{NH,18}$ 7
2.4	2.75 d,d $J_{16,15}$ 12 $J_{16,7}$ 10			5.88 s	3.52 s (CO_2Me)
	2.75 d,d $J_{16,15}$ 11 $J_{16,7}$ 7			5.9 s	3.42 s (CO_2Me)

intensity in the cases of **7** and **18**. Off-resonance decoupling experiments reveal the presence of four singlets (C(1), C(3), C(4) and C(17)), five doublets in the olefinic-aromatic region (C(2), C(5), C(6), C(8) and C(9)), four doublets in the aliphatic region (C(7), C(10), C(15) and C(16)) and nine triplets (C(11), C(12), C(13), C(14), C(18), C(19), C(20), C(21) and C(22)). There are no signals of quaternary carbon atoms in the aliphatic region. Assignments of signals within these groups are given in Table 2. The spectral pattern in the low-frequency region (20–40 ppm) is very similar for **7** and **18** which both have a *trans*-octahydronaphthalene system, but differs considerably in **2** with a *cis*-skeleton. Further differences in the chemical shifts of the three stereoiso-

Table 2. *^{13}C-NMR. data*[a]) *of the cyclostachines and the synthetic amide* **18**

	C(17)	C(3,4)	C(1)	C(6,8,9)	C(2,5)	C(22)	C(7,16)	C(18,21)	C(10,15)	C(11,12,13,14) C(19,20)	
	173.6	147.7	138.7	133.4	108.4	101.0	47.2	46.4	36.6	30.6	26.0
2		146.3		128.5	108.1		46.8	45.4	35.6	28.8	24.3
				121.0						26.4	22.1
	170.2	147.3	135.1	133.3	110.0	100.9	49.9	46.4	36.6	42.2	26.4(2)
7		146.5		127.5	107.7		42.6	45.5	30.7	33.3	24.3
				122.4						26.9	
	173.0	147.5	139.7	134.1	108.7	100.8	49.4	47.4	36.6	38.0	26.4(2)
18		145.8		125.4	108.1		44.0	45.6	30.6[b])	33.3	24.4
				121.0						26.9	

[a]) δ [ppm], $\pm$ 0.2 ppm.
[b]) Tentative assignment, doublet-structure not proven.

mers are observed, as expected, in the resonances of C(15), C(16) and C(7). In all three compounds the diastereotopic carbons C(18) and C(21) exhibit different resonance frequencies.

Cyclostachines A and B evidently arise by intramolecular cyclisation of the triene-amide **9** and cyclopiperstachine from piperstachine (**1**). These three compounds have been isolated from the *cold* hexane extract and evaporation of the extract *in vacuo*. Since the thermal cyclisation of piperstachine (**1**) seems to require a temperature of 110°, the three new compounds reported here do not appear to be artefacts formed in the isolation stage.

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Experimental Part

UV. and IR. spectra were determined on *Beckman* model DK-2A and *Perkin-Elmer* model 421 spectrophotometers. MS. were recorded on an *Atlas Varian* Mat CH-7 spectrometer using direct inlet system. ^{13}C-NMR. spectra were measured on an XL-100-15 spectrometer at 25.2 MHz in the pulsed mode, ^{1}H-NMR. spectra on a *Varian* A-60 instrument, including double resonance experiments on an HA-100 instrument. Deuteriochloroform as solvent and tetramethylsilane as an internal reference were used for ^{13}C-NMR. spectra. Chemical shifts are given in δ-values (ppm), coupling constants in Hz.

Isolation. The powdered stem of *Piper trichostachyon* (6 kg) was extracted with hexane (3 × 30 l) by cold percolation. The extract was evaporated *in vacuo* to yield a greenish oil (80 g). This was dissolved in hexane and chromatographed over a column of neutral alumina (2 kg) and gradient-eluted with hexane, hexane/C_6H_6, C_6H_6/$CHCl_3$ and $CHCl_3$/MeOH. Fractions (300 ml) were collected and monitored by TLC. Fractions 16–20 (eluant: hexane/C_6H_6 1:3) gave a yellow oil (15 g) which was rechromatographed over silica gel in C_6H_6 to give cyclopiperstachine (**10**) (70 mg), colourless needles (from CH_2Cl_2/hexane), m.p. 220°, TLC., silica gel, $CHCl_3$/MeOH 99:1,

R_f 0.65. – UV. (λ_{max}^{EtOH}): 236, 286 nm (log ε 3.67, 3.62). – IR. (ν_{max}^{KBr}): 3340, 1645, 1535, 1505, 1490, 1470, 1445, 1390, 1370, 1360, 1350, 1300, 1260, 1215, 1200, 1190, 1160, 1105, 1045, 930, 880, 835, 805 cm^{-1}. – ^1H-NMR. see Table 1. – MS. (*m/e*): 355 (M^+, 50%), 272 (12), 256 (38), 255 (68), 254 (57), 240 (10), 220 (22), 162 (20), 148 (34), 141 (15), 135 (100), 121 (50), 115 (25).

$C_{22}H_{29}NO_3$ (355,5) Calc. C 74.3 H 8.2 N 3.9% Found C 74.2 H 8.4 N 4.2%

Fractions 28–35 (eluant: C_6H_6) yielded colourless cubes of cyclostachine A (**2**) (1.2 *g*), m.p. 136–138° (from ether/hexane), TLC., silica gel, $CHCl_3$/MeOH 99:1, R_f 0.5. – UV. (λ_{max}^{EtOH}): 235, 287 nm (log ε 3.65, 3.62). – IR. (ν_{max}^{KBr}): 1640, 1500, 1480, 1430, 1380, 1245, 1190, 1115, 1100, 1035, 970, 925, 900, 880, 870, 832, 812 800, 758, 750, 730, 720 cm^{-1}. – ^1H-NMR. see Table 1. – MS. (*m/e*): 353 (M^+, 100%), 270 (45), 255 (44), 254 (90), 240 (18), 218 (45), 212 (8), 211 (15), 204 (8), 181 (6), 169 (6), 153 (10), 152 (10), 148 (12), 141 (14), 135 (78), 128 (20), 115 (25), 103 (12), 98 (95), 91 (24), 77 (22), 70 (24), 56 (35), 55 (92).

$C_{22}H_{27}NO_3$ (353.5) Calc. C 74.8 H 7.7 N 4.0% Found C 74.7 H 7.9 N 3.8%

Fractions 39–41 (eluant: C_6H_6) yielded cyclostachine B (**7**) (250 mg), colourless needles (from CH_2Cl_2/hexane), m.p. 135–136°. – TLC., silica gel, $CHCl_3$/MeOH 99:1, R_f 0.6. – UV. (λ_{max}^{EtOH}): 236, 287 nm (log ε 3.73, 3.62). – IR. (ν_{max}^{KBr}): 1640, 1500, 1490, 1480, 1370, 1295, 1275, 1240, 1210, 1190, 1165, 1120, 1100, 1090, 1070, 1045, 995, 965, 940, 900, 890, 880, 828, 810, 790, 785, 735, 725, 700 cm^{-1}. – ^1H-NMR. see Table 1. – MS. (*m/e*): 353 (M^+, 86%), 270 (8), 255 (35), 254 (21), 240 (14), 218 (100), 192 (11), 161 (13), 152 (28), 139 (22), 135 (40), 98 (35), 91 (21), 70 (30), 55 (35).

$C_{22}H_{27}NO_3$ (353.5) Calc. C 74.8 H 7.7 N 4.0% Found C 74.5 H 8.0 N 4.0%

Hydrolysis of cyclostachine **A**. Cyclostachine A (**2**) (100 mg) was heated with 6 N HCl (4.5 ml) in a sealed tube at 100–110° for 16 h. The solution was extracted with CH_2Cl_2 to remove nonbasic impurities. The aqueous acid solution was evaporated *in vacuo*, the residue treated with a few drops of methanolic NH_3 and the solution used as such for GC. Part of the solution was adsorbed on chromosorb for mass spectrum. The product was identical with authentic pyrrolidine in retention time in GC. (Instrument: *Varian* VA 2740) column chromosorb 103, length of column: 6', internal diameter 3 mm, hydrogen flow rate 40 ml/min, column temp. 159°, injection block temp. 270°, detector temp. 300°, detector: FID).

Its MS., *m/e* 71 (M^+, 14%), 70 (18), 43 (82), 42 (12), 28 (100), was identical with the reported MS. of pyrrolidine [5].

Dihydrocyclostachine A (**3**). A solution of cyclostachine A (100 mg) in EtOH (15 ml) was shaken with H_2 at atmospheric pressure over Pd/C catalyst (5%, 100 mg). The solution was filtered, evaporated and the gum crystallised from ether/hexane to yield dihydrocyclostachine A (40 mg), m.p. 165°. – UV. (λ_{max}^{EtOH}): 233, 287 nm (log ε 3.66, 3.58). – MS. (*m/e*): 355 (M^+, 58%), 272 (20), 257 (32), 256 (95), 247 (16), 246 (23), 242 (15), 220 (28), 175 (15), 162 (20), 148 (44), 135 (100), 131 (18), 113 (60), 103 (17), 98 (60), 70 (35).

$C_{22}H_{29}NO_3$ (355.5) Calc. C 74.3 H 8.2 N 3.9% Found C 74.5 H 8.5 N 4.3%

Diol **4**. A solution of cyclostachine A (190 mg) in dioxane (5 ml) was treated with OsO_4 (200 mg) and pyridine (0.2 ml) and left for 40 h at room temp. The complex was decomposed with H_2S, the solution filtered and the filtrate evaporated *in vacuo*. Crystallisation of the residue from EtOAc/hexane yielded the diol **4** (140 mg), m.p. 167°. – ^1H-NMR. ($CDCl_3$): 6,75 (3 H, *m*); 5.88 (2 H, *s*). – MS. (*m/e*): 369 ($M^+ - H_2O$, 100%), 340 (25), 286 (25), 270 (85), 253 (40), 246 (60), 193 (74), 175 (40), 135 (75), 98 (52).

$C_{22}H_{29}NO_5$ (387.5) Calc. C 68.2 H 7.5% Found C 68.5 H 7.7%

Dialdehyde **5**. A solution of the above diol (250 mg) in MeOH (10 ml) was treated with a solution of sodium metaperiodate (300 mg) in H_2O) in H_2O (10 ml) and allowed to stand at room temp. for 20 h. Dilution with H_2O, extraction with $CHCl_3$ and chromatography of the product over silica gel in $CHCl_3$/EtOAc 1:1 gave the dialdehyde **5** (55 mg), m.p. 116° (from EtOAc/hexane). – IR. (ν_{max}^{KBr}): 2830, 2700, 1710 cm^{-1}. – ^1H-NMR. ($CDCl_3$): 9.74 (1H, *t*, $\bar{J} \approx 1.5$); 9.67 ppm (1H, *d*,

$J = 2$); 6.6–6.85 (3H, *m*). – ^{13}C-NMR. ($CDCl_3$): 205.3, 199.2 (2 —CHO). – MS. (*m/e*): 385 (M^+, 5%), 339(35), 274(35), 257(26), 246(74), 240(72), 204(63), 175(50), 135(54), 98(100).

$C_{22}H_{27}NO_5$ (385.5) Calc. C 68.5 H 7.1% Found C 68.5 H 7.4%

$LiAlH_4$-reduction of cyclostachine A. A solution of cyclostachine A (500 mg) in dry ether (40 ml) was added with stirring to $LiAlH_4$ (500 mg) in ether (40 ml) under N_2. The mixture was stirred at 50° for 5 h, cooled, decomposed with 20% H_2SO_4 (50 ml) and left at room temp. for 2 days. The solid was filtered off, washed with ether and a little H_2O, and crystallized from MeOH to yield the sulfate of amine **6** (100 mg), m.p. 110°. – UV. (λ_{max}^{EtOH}): 235, 287 nm (log ε 3.62, 3.58).

$C_{22}H_{29}NO_2 \cdot H_2SO_4 \cdot {}^1/_2\,H_2O$ (446.6) Calc. C 59.2 H 7.2 N 3.1 S 7.2%
Found „ 59.3 „ 7.6 „ 3.3 „ 7.4%

The free amine, obtained as a gum, showed mass spectral peaks at *m/e* 339(M^+, 10%), 268(8), 254(7), 225(7), 211(15), 198(7), 186(28), 181(8), 169(8), 165(10), 153(16), 152(16), 148(10), 141(24), 135(60), 131(16), 129(22), 128(32), 127(16), 115(42), 103(32), 91(42), 85(72), 84(100), 77(38), 70(36), 55(70).

Dihydrocyclostachine B (**8**). Cyclostachine B (150 mg) in EtOH (25 ml) was hydrogenated at atmospheric pressure over Pd/C catalyst (10%, 150 mg) to yield dihydrocyclostachine B (40 mg), m.p. 95° (from ether/hexane). – UV. (λ_{max}^{EtOH}): 233, 287 nm (log ε 3.69, 3,61). – MS. (*m/e*): 355 (M^+, 30%), 260 (15), 256 (17), 247 (5), 246 (7), 242 (7), 220 (46), 161 (12), 148 (28), 135 (52), 131 (12), 113 (100), 98 (35), 70 (15).

$C_{22}H_{29}NO_3$ (355.5) Calc. C 74.3 H 8.2 N 3.9% Found C 74.2 H 8.5 N 4.3%

Synthesis. – *Cyclisation of methyl 11-(3,4-methylenedioxyphenyl)-undeca-2,8,10-*trans, trans, trans-*trien-oate* (**11**). A solution of the triene ester **11** (1.6 g) in xylene (30 ml) was refluxed under N_2 for 3 h, evaporated *in vacuo* and the residue crystallized from ether/hexane to yield the ester **12** (0.6 g), m.p. 136°. – UV. (λ_{max}^{EtOH}): 235, 286 nm (log ε 3.60, 3.64). – IR. (ν_{max}^{KBr}): 1720 cm^{-1}. – ^{1}H-NMR. see Table 1. – MS. (*m/e*): 314 (M^+, 54%), 254 (100), 212 (18), 211 (24), 206 (22), 181 (40), 173 (44), 169 (36), 148 (90), 135 (95), 131 (90), 119 (85), 115 (72), 103 (45), 91 (82).

$C_{19}H_{22}O_4$ (314.4) Calc. C 72.6 H 7.1% Found C 72.4 H 7.3%

Chromatography of the product from the mother liquor of the above ester over alumina in hexane yielded the isomer **13** (0.3 g), m.p. 87° (from ether/hexane). – UV. (λ_{max}^{EtOH}): 237, 286 nm (log ε 3.65, 3.60). – IR. (ν_{max}^{KBr}): 1725 cm^{-1}. – ^{1}H-NMR. see Table 1. – MS. (*m/e*): 314 (M^+, 100%), 255 (28), 254 (62), 240 (6), 232 (20), 211 (8), 206 (20), 187 (32), 173 (55), 157 (28), 148 (65), 135 (86).

$C_{19}H_{22}O_4$ (314.4) Calc. C 72.6 H 7.1% Found C 72.6 H 7.3%

Hydrolysis of the ester (**12**). – a) *Acid* **14**. The ester **12** (0.3 g) in dioxane (8 ml) was refluxed with KOH (3 g) in MeOH (2 ml) and H_2O (2 ml) for 3 h, evaporated *in vacuo*, diluted with H_2O and extracted with ether. The aq. solution was acidified and extracted with ether to yield the acid **14** (250 mg), m.p. 140–143° (from ether/hexane). – UV. (λ_{max}^{EtOH}): 235, 286 nm (log ε 3.72, 3.65). – IR. (ν_{max}^{KBr}): 1702 cm^{-1}. – MS. (*m/e*): 300 (M^+, 100%), 255 (25), 254 (24), 218 (16), 206 (24), 192 (32), 187 (20), 173 (46), 148 (85), 135 (60), 115 (30).

$C_{18}H_{20}O_4$ (300.3) Calc. C 72.0 H 6.7% Found C 72.0 H 7.0%

Re-esterification of the acid with ethereal diazomethane yielded the ester **12** identified by mixed m.p. and IR. spectrum.

b) *Acid* (**15**). The ester **12** (2 g) in 2-ethoxy-ethanol (30 ml) was refluxed with KOH (12 g) and H_2O (3 ml) for 45 min. The solution was evaporated *in vacuo*, the residue diluted with H_2O and worked-up as usual. The gummy acidic product was chromatographed over silica gel in hexane/EtOAc 9:1. The initial fractions yielded the acid **14** (0.5 g). Later fractions gave the isomer **15** (0.2 g), m.p. 158° (from ether/hexane). – UV. (λ_{max}^{EtOH}): 208, 257, 295 nm (log ε 4.40, 3.95, 3.72). – IR. (ν_{max}^{KBr}): 1705 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$ + DMSO-d_6): 10.33 (1H, *s*, OH); 6.7–6.9 (3 H, *m*);

6.07 (1 H, *t*, $J = 3$); 5.9 (2 H, *s*); 3.36 (1 H, br. *s*); 2.2 (4 H, br. *s*); 1.5 (8 H, br. *s*). – MS. (*m/e*): 300 (M^+, 100%), 255 (28), 218 (15), 206 (30), 192 (28), 187 (21), 173 (39), 162 (20), 148 (52), 135(45).

$C_{18}H_{20}O_4$ (300.3) Calc. C 72.0 H 6.7% Found C 72.1 H 7.0%

Hydrolysis of the ester (**13**). – a) *Acid* (**16**). The ester **13** (100 mg) was refluxed mildly for 2 h with KOH (150 mg) in MeOH (2 ml) and H_2O (0.2 ml) to yield the acid **16** as a gum. – UV. (λ_{max}^{EtOH}): 236, 286 nm (log ε 3.69, 3.64). – ^{1}H-NMR. ($CDCl_3$): 10.51 (1 H, *s*, OH); 6.7 (3 H, br. *s*); 5.92 (2 H, *s*); 5.65 (2 H, br. *s*); 3.75 (1 H, br. *s*); 2.75 (1 H, $d \times d$, $J = 8$ and 10).

b) *Acid* (**17**). The ester **13** (1 g) in dioxane (5 ml) was refluxed for 4 h with KOH (3 g), MeOH (5 ml) and H_2O (3 ml). The crude acid obtained was chromatographed over silica in hexane/EtOAc 9:1 to yield the acid **17** (0.5 g), m.p. 180° (from CH_2Cl_2/hexane). – UV. (λ_{max}^{EtOH}): 236, 286 nm (log ε 3.67, 3.65). – IR. (ν_{max}^{KBr}): 1698 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$): 11.1 (1 H, br. *s*, OH); 6.77 (3 H, *s*); 5.93 (2 H, *s*); 5.7 (2 H, br. *s*); 3.75 (1 H, br. *s*); 2.6 (1 H, br. *s*). – MS. (*m/e*): 300 (M^+, 72%), 255 (16), 254 (15), 240 (6), 192 (54), 187 (10), 173 (16), 148 (100), 135 (35), 115 (15).

$C_{18}H_{20}O_4$ (300.3) Calc. C 72.0 H 6.7% Found C 72.1 H 7.0%

Cyclostachine A (**2**). The acid **14** (80 mg) in C_6H_6 (5 ml) was refluxed mildly with oxalyl chloride (0.3 ml) for 1 h. The solution was evaporated *in vacuo*, dried well, redissolved in C_6H_6 (5 ml) and treated with pyrrolidine (1 ml). The solution was left overnight at room temp., washed (dil. HCl, H_2O), dried (Na_2SO_4) and evaporated. Crystallization of the residue from ether/hexane yielded the amide **2**, identical (mixed m.p., UV., IR., ^{1}H-NMR. and MS.) with cyclostachine A.

$C_{22}H_{27}NO_3$ (353.5) Calc. C 74.8 H 7.7 N 4.0% Found C 74.9 H 7.9 N 4.0%

Cyclostachine B (**7**). The acid **16** (150 mg) in C_6H_6 (3 ml) was refluxed for $^1/_2$ h with oxalyl chloride (0.5 ml). The acid chloride obtained was treated with excess pyrrolidine as above to yield, after chromatography over alumina in hexane/CH_2Cl_2 (8:2), the amide **7** (50 mg), m.p. 135–136° (from ether/hexane), identical (mixed m.p., UV., IR., ^{1}H-NMR. and MS.) with cyclostachine B.

Amide **18**. The acid **17** (250 mg) in C_6H_6 (5 ml) was refluxed mildly for $1^1/_2$ h with oxalyl chloride (1 ml). The acid chloride obtained was condensed with pyrrolidine (1 ml) to yield the amide **18** (150 mg), m.p. 150° (from CH_2Cl_2/hexane). – UV. (λ_{max}^{EtOH}): 235, 286 nm (log ε 3.71, 3.65). IR. (ν_{max}^{KBr}): 1635 cm^{-1}. – ^{1}H-NMR. see Table 1. – MS. (*m/e*): 353 (M^+, 86%), 270 (30), 255 (30), 254 (44), 246 (10), 240 (18), 218 (76), 135 (100).

$C_{22}H_{27}NO_3$ (353.5) Calc. C 74.8 H 7.7 N 4.0% Found C 74.5 H 8.0 N 4.3%

Cyclopiperstachine (**10**). – a) The acid **14** (250 mg) in C_6H_6 (5 ml) was refluxed mildly with oxalyl chloride (1 ml) for 1 h and the acid chloride obtained was treated with isobutylamine (1 ml) to yield the amide **10** (180 mg), m.p. 220° (from CH_2Cl_2/hexane) identical (mixed m.p., UV., IR., ^{1}H-NMR. and MS.) with cyclopiperstachine. b) A solution of piperstachine (**1**) (0.3 g) in xylene (10 ml) was refluxed for 3 h under N_2, evaporated *in vacuo* and the residue chromatographed over alumina in hexane/CH_2Cl_2 (9:1) to yield the amide **10** (0.1 g), m.p. 220°, identical (mixed m.p., IR. spectrum) with natural cyclopiperstachine.

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