Isolation, Characterization, and Synthesis of *trans*-Pilosine Stereoisomers Occurring in Nature. Circular Dichroism and Mass Spectral Studies[1]

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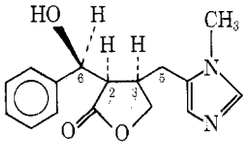
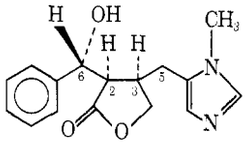
"Pilosine" of $[\alpha]^{20}D$ +83.9° is shown to be a 1:1 mixture of pilosine and isopilosine. Data on pure pilosine are described. The isolation and assignment of structure of a new pilosine constituent in *Pilocarpus jaborandi* are herein presented. From circular dichroism measurements it is concluded that pilosine, isopilosine, and epiisopilosine are of 2*R*,3*R*,6*S* (**1**), 2*S*,3*R*,6*S* (**4**), and 2*S*,3*R*,6*R* configurations (**3**), respectively. Optimal conditions for the production of **3**, **4**, and the fluoro derivatives (**3a** and **3b**), *via* aldol condensation between benzaldehyde, 2- and 4-fluorobenzaldehydes, and (3*R*)-pilosinine, in the presence of lithium methoxide, have been worked out. Effects of stereochemistry on trends of mass spectral fragmentations are discussed in terms of abundances of M − H_2O and *m/e* 223 ions being higher in the spectrum of the cis (**1**) than in that of the trans (**4**) isomer and in terms of [M − 1]/[M] ratios.

Until recently, only two of the four theoretically possible isomers (**1**–**4**) of (3*R*)-pilosine, namely, pilosine and isopilosine, were known to occur in nature. The synthesis[2] and the occurrence in nature of a 6 epimer of isopilosine has been reported lately.[1,3] According to Oberhansli,[4] pilosine, isopilosine, and epiisopilosine are assigned the 2*R*,3*R*,6*R*, 2*S*,3*R*,6*R*, and 2*S*,3*R*,6*S* configurations, respectively.



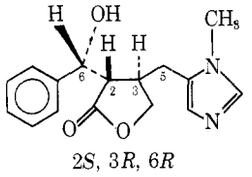
2*R*, 3*R*, 6*S*

1



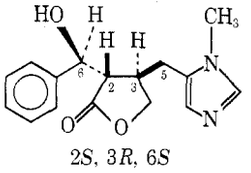
2*R*, 3*R*, 6*R*

2



2*S*, 3*R*, 6*R*

3



2*S*, 3*R*, 6*S*

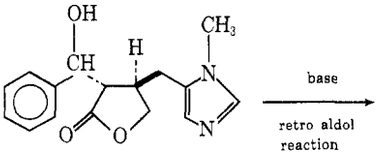
4

This paper gives an account of the isolation, characterization, and synthesis of the newly discovered naturally occurring imidazole alkaloid from a *Pilocarpus* species, shown to be epiisopilosine. Evidence is also adduced showing that the common crystalline pilosine of mp 176–178°, $[\alpha]D$ +83.9°[5], is in fact a "molecular compound" comprised of pilosine and isopilosine in equimolar ratio.

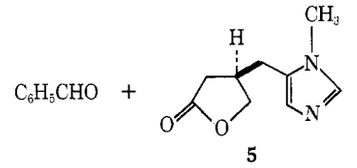
Later in this paper we shall show that carbon 6 in (−)-epiisopilosine should be assigned the *R* configuration rather than the *S* configuration as suggested above.[4] Accordingly, (+)-pilosine, (+)-isopilosine, and (−)-epiisopilosine should be assigned the 2*R*,3*R*,6*S* (**1**), 2*S*,3*R*,6*S* (**4**), and 2*S*,3*R*,6*R* (**3**) structures, respectively. This sense will be used throughout this paper when referring to pilosine.

The nmr spectrum (in DMSO) of "pilosine" prepared according to literature[5] was found to be complex. It appeared as spectra of two closely related compounds superimposed one on another, suggesting that the sample is in fact comprised of a 1:1 isomeric mixture (see Table I). To attain the desired genuine pilosine we set out to separate the two isomers, by first converting "pilosine" into the respective mixture of nitrates and then allowing it to undergo partial crystallization. This resulted in quick separation of isopilosine nitrate, leaving in solution the highly soluble pilosine nitrate. After neutralization and removal of precipitating "pilosine," the free base of genuine pilosine could be induced to crystallize as a dihydrate melting at 102–104°, $[\alpha]^{20}D$ +118°. The anhydrous form of pilosine was obtained by heating the dihydrate to 105° under high vacuum. When mixed with an equimolar quantity of isopilosine, the resulting material behaved as a typical "molecular compound" rather than a simple mixture and displayed identity in all respects with the starting "pilosine."

In the course of experiments aimed at extracting isopilosine from large leaves of *Pilocarpus jaborandi* we isolated a new constituent in 8.5% of the total alkaloid mixture which is in the range of 0.8–1.3% of the dried leaves. Other components characterized by tlc and spectroscopic methods were pilocarpine (70%) and isopilosine (10–18%). Whereas isopilosine gives on tlc plate a violet color on reaction with iodoplatinic acid, the new substance yields a blue color with the same reagent. Typically, pilosine exhibits double ir absorption at 1753 and 1765 cm^{-1}, whereas isopilosine and the new substance show single bands only, at 1765 and 1753 cm^{-1}, respectively. The uv absorption at 262.5 mμ is observed in the new substance only. The crystalline form of the new substance was analyzed as a $C_{16}H_{18}N_2O_3$ compound, and displayed an optical rotation of similar value but of opposite sign to that of isopilosine. On treatment with aqueous 20% KOH the new



(+)-isopilosine or (−)-epiisopilosine



C_6H_5CHO +

5

(+)-(3*R*)-pilosinine

compound underwent aldol cleavage to yield pilosinine (**5**) and benzaldehyde. These data strongly suggested that the new substance must be a 6 epimer of isopilosine. This was

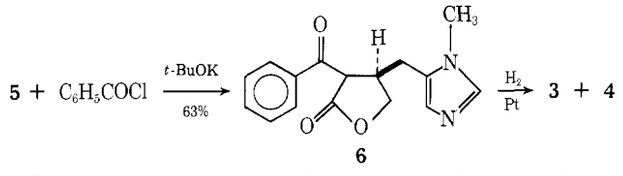
Table I
Characteristic Data of Isomeric Pilosines and Derivatives

Alkaloid	Mp, °C	$[\alpha]^{25}D$,[a] deg	Nmr, τ[b]	
"Pilosine" according to literature (ref 5)	176–178	+84	–CH(OH) –OCH_2– –NCH_3	d 4.82, d 5.0 d 6.30, d 6.08; d 5.91, d 5.50 s 6.72, s 6.55
Pilosine	172	+136.8	–CH(OH) –OCH_2 –NCH_3	d 4.82, J = 3 Hz d 6.30, d 5.50 s 6.72
Isopilosine	182.0–182.5	+33.5	–CH(OH) –OCH_2– –NCH_3	d 5.0, J = 3 Hz d 6.08, J = 5 Hz d 5.91, J = 5 Hz s 6.55
Epiisopilosine	182–184	−44.8	–CH(OH) –OCH_2– –NCH_3	d 4.76, J = 2 Hz d 6.08, J = 6.5 Hz d 5.64; J = 7.0 Hz s 6.95
6-Deoxypilosine	136–138	+98.3 +73.2[c]	–OCH_2– –NCH_3	d 5.75, d 6.20 s 6.70
o-Fluoroepiisopilosine	207–210	−39.9	–CH(OH)– –OCH_2– –NCH_3	d 4.56 d 6.02; J = 27 Hz d 5.58 J = 27 Hz s 6.80
p-Fluoroepiisopilosine	165–168	−28.2	–CH(OH)– –OCH_2– –NCH_3	d 4.75 d 6.08; J = 27 Hz d 5.65 J = 27 Hz s 6.83

[a] In ethanol (c 1). [b] In dimethyl sulfoxide, 60 MHz, Me_4Si as internal standard. [c] In water (c 1).

finally established by its comparison with a sample of epiisopilosine produced by synthesis.

A two-step synthesis of (−)-epiisopilosine in 17% overall yield has been recently reported by Link and Bernauer.[2] It involves an initial benzoylation of **5** in a stereoselective manner to yield **6**, followed by catalytic reduction in a nonstereoselective manner to yield (+)-isopilosine and (−)-epiisopilosine, each in approximately 25% yield.



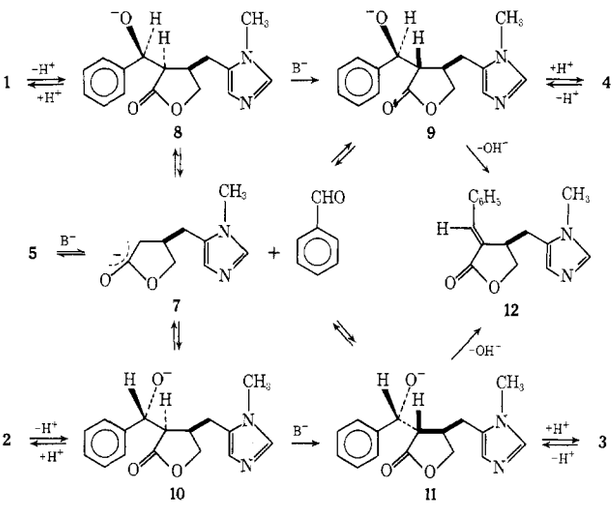
6

As part of a project aimed at total synthesis of pilosine and isopilosine we set to study the aldol reaction between **5** and benzaldehyde. This involved a study of the four reaction variables, catalyst, solvent, temperature, and time, on product distribution. Early in this study it became apparent that 20% alkali hydroxide favored the retrograde process. Alkali metal hydrides, on the other hand, although manifesting high potency in pushing the aldol reaction forward (**7** → **8** + **9** → **4** and **7** → **10** + **11** → **3**), promote also the concurrent path which involves a loss of hydroxide anion from the presumed anionic species **9** and **11** to yield anhydropilosine (**12**) (see Scheme I).

We then turned our attention to the use of alkali alkoxides in alcohols to catalyze this reaction. The total yield of pilosines was found to increase in the order $Mg(OCH_3)_2$ < $LiOCH_3$ < $NaOCH_3$ and, in parallel, the isomer ratio epiisopilosine/isopilosine increased from 0.8 to 1.6 to 2.7, reaching a top value of 14.0 in the case of potassium *tert*-butoxide in *tert*-butyl alcohol (compare expt 15 in Table II). The latter, however, is characterized by lower yields of pilosine stereoisomers, and by a significant increase in yields of **12** at the expense of the former (compare expt 12, 15, and 19 in Table II).

Analysis of product distribution as a function of reaction variables indicates that the ratio **12**/(**3** + **4**) is sensitive to proton concentration in the medium. For example, in presence of $LiOCH_3$–*t*-BuOH the ratio (**12**/(**3** + **4**) increases with the progress of reaction, but decreases remarkably on adding small amounts (5%) of methanol (expt 11). This suggests that the tendency of the less basic alkoxides, **9** and **11**, to lose a hydroxide ion (**9** → **12**, **11** → **12**) or to pick up a proton (**9** → **4**, **11** → **3**) is determined by the acid-base equilibrium existing in the system.

Scheme I



From product study it appears that the formation of epiisopilosine is governed by kinetic factors and isopilosine seems to be the thermodynamically controlled product. The nonobservance of the 2,3-cis isomers, **1** and **2**, among the reaction products is probably due to their instability in basic media; they are isomerized to the 2,3-trans isomers as soon as formed, as delineated in Scheme I (**8** → **9** → **4** and **10** → **11** → **3**).

With this background, we tried to react *o*- and *p*-fluorobenzaldehyde with **5** in presence of lithium methoxide in *t*-BuOH–MeOH at room temperature. In each case the

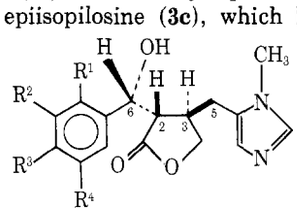
Table II
Effects of Reaction Conditions on Product Distribution Resulting from Base-Catalyzed Condensation between (+)-Pilosinine (5) and Benzaldehyde

Expt	Catalyst: Formula	Catalyst: Molar equiv	Solvent	Temp, °C	Time, hr	Product distribution, %: 5	4[a]	3[b]	12
1	KOH	0.5	H_2O	80	96	70	15		15
2		0.5	Dry EtOH	80	96	70	15		15
3	$Mg(OMe)_2$	2.0	MeOH	25	2	20	36	30	14
4		4.0	MeOH	25	48	10	80		10
5	LiOMe	2.0	MeOH	5	48	10	70		20
6		2.0	MeOH	25	0.5	75	20		5
7		2.0	*t*-BuOH	60	2	100			
8		2.0	*t*-BuOH	85	0.25	75	20		5
9		2.0	*t*-BuOH	85	2	70	20		10
10		2.0	*t*-BuOH	85	5	30	30		40
11		2.0	5% MeOH–95% *t*-BuOH	25	2	9.5	31	51	8
12	NaOMe	2.0	*t*-BuOH	25	2	0	26.5	73.4	0
13		1.0	*t*-BuOH	80	96	70	30		0
14		1.0	C_6H_6	80	168	30	40		30
15	$KOC(CH_3)_3$	2.0	*t*-BuOH	25	2	0	5	70	25
16	LiH	1.2	*t*-BuOH	40	0.5	0	25	20	55
17	NaH	1.2	DMSO	25	3	0	20	17	63
18		1.0	DMF	−5	2	0	70		30
19		1.0	*t*-BuOH	25	2	0	65		35
20		1.0	*i*-AmOH	5	18	10	60		30
21		1.0	C_7H_8	25	24	100	0		0

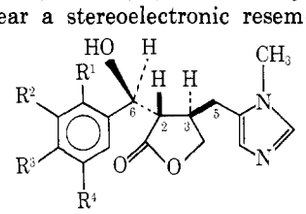
[a] Isopilosine. [b] Epiisopilosine.

stereoisomer which displays a strong negative Cotton effect at around 220 mμ could be isolated from the reaction mixture, and consequently these isomers were assigned the 6*R* configuration (see Experimental Section).

For biological testing we became interested in preparing 3′,4′,5′-trimethoxyisopilosine (**4c**) and 3′,4′,5′-trimethoxyepiisopilosine (**3c**), which bear a stereoelectronic resem-



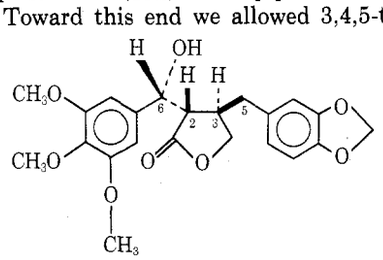
3a–c



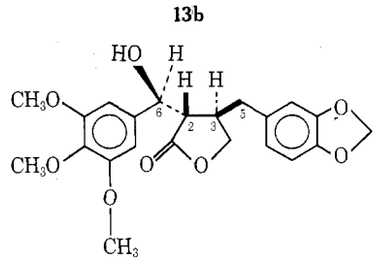
4a–c

a, $R^1 = F$; $R^2 = R^3 = R^4 = H$
b, $R^3 = F$; $R^1 = R^2 = R^4 = H$
c, $R^1 = H$; $R^2 = R^3 = R^4 = OCH_3$

blance to podorhizol (**13a**) and epipodorhizol (**13b**), respectively. Toward this end we allowed 3,4,5-trimethoxy-



2*S*, 3*S*, 6*R*,
13b

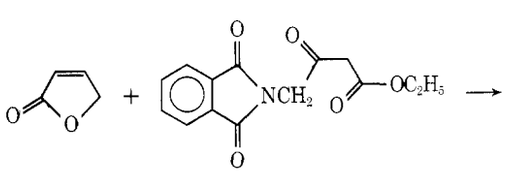


2*S*, 3*R*, 6*S*
13a

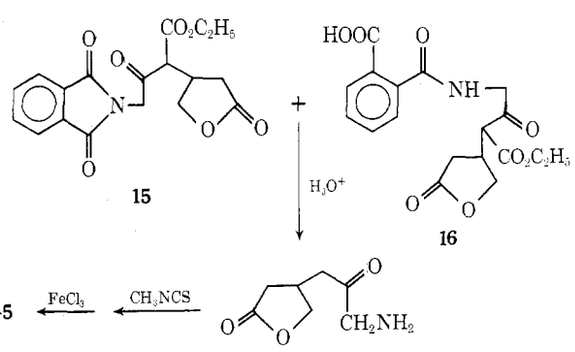
benzaldehyde to react with (3*R*)-pilosinine under various aldol reaction conditions but as yet with no success.

The various pathways to total synthesis of racemic (*rac*-**5**) and (3*R*)-pilosinine (**5**)[2] are well documented.[6-8] They are all characterized by poor yields. The key substance toward *rac*-**5** appears to be aminomethyl homopilosinyl ketone (**17**), which is amenable to a smooth reaction with methyl isothiocyanate followed by oxidation with ferric chloride.[7] We succeeded here in producing **17** in higher yields by allowing γ-crotonolactone to enter into Michael reaction with ethyl phthalimidoacetoacetate (**14**) in the presence of sodium ethoxide, followed by acid hydrolysis of the adduct mixture **15** and **16**.

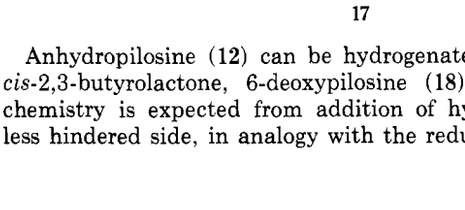
Since the optical resolution of *rac*-**5** is known, we regard the sequence **14** → *rac*-**5** → **5** → **3** + **4** described here as a new total synthesis of pilosines.



14



15 **16**



17

Anhydropilosine (**12**) can be hydrogenated to yield the *cis*-2,3-butyrolactone, 6-deoxypilosine (**18**). This stereochemistry is expected from addition of hydrogen to the less hindered side, in analogy with the reduction of anhy-

Table III
Relative Abundances of Prominent Peaks in the Mass Spectral Fragmentation of Isomeric Pilosines and Derivatives

m/e	Pilosine, %	Isopilosine, %	Epiiso-pilosine, %	*o*-Fluoroepiiso-pilosine, %	*p*-Fluoroepiiso-pilosine, %	Anhydro-pilosine, %
83	28.7	48.3	35.1	25.2	22.1	
95	100	100	100	100	100	100
96	47.0	57.2	53.2	45.0	42.0	9.7
105	24.5	28.6	25.6			
106	20.4	27.5	24.1			
123	20.4	12.1	11.0	37.9	31.5	
124				28.0	26.6	
137	10.2	4.4	8.3	14.9	3.9	
138	12.2	5.5	9.6	14.3	5.5	
180	6.1	6.6	8.3	7.7	7.7	
223	6.1	1.1	2.7			
241				2.9	1.1	
M − H_2O	6.1	2.2	5.4	6.7	2.8	5.1
M·$^+$	5.1	4.4	8.9	6.1	3.3	
[M − 1]/[M]	0.27	0.43	0.30			

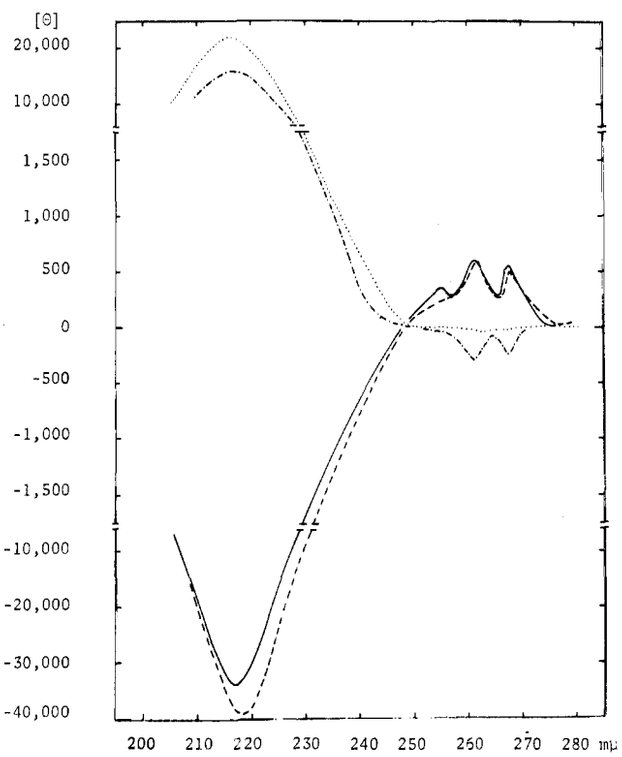
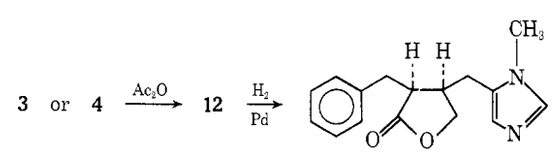


Figure 1. CD spectra of pilosine (. . . . .), isopilosine (- . - . - . -), natural epiisopilosine (——), and synthetic epiisopilosine (- - - - -) in ethanol.

dropodorhizol to dihydroanhydropodorhizol[9] and that of hibalactone to isohinkinin.[10,11]



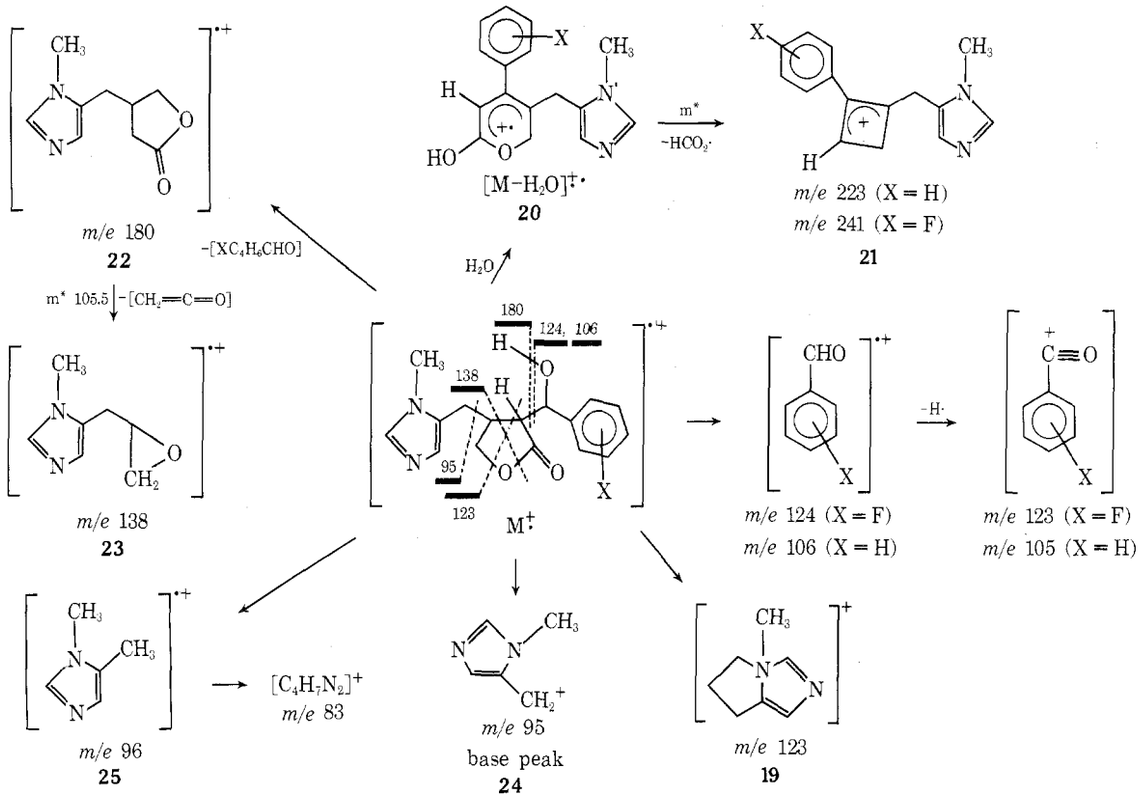
18

Circular Dichroism. The most striking difference between isopilosine and epiisopilosine is reflected in the signs of their optical rotations. It is positive for the former and negative for the latter. Furthermore, the circular dichroism curves of the two isomers are of the mirror image type (see Figure 1). Since these mirror image type bands around 220 and 260 mμ are associated with the phenyl absorptions, this indicates that the isomers are indeed epimeric at carbon 6. Moscowitz, Rosenberg, and Hansen[12a] have established the existence of optically active aromatic absorption bands for aromatic amino acids: the sign of the high-wavelength transition (^1L_b) of the phenyl group around 270 mμ is negative and the sign at the low-wavelength phenyl transition (^1L_a) around 220 mμ is positive for compounds with *S* configuration.[13] Following this consideration, pilosine and isopilosine, which exhibit positive dichroic bands in the low-wavelength region, should be assignd the 6*S* configuration and should therefore be formulated as structures **1** and **4**, respectively. Epiisopilosine, on the other hand, should be assigned the 2*S*,3*R*,6*R* configuration and be represented by formula **3**. Indeed, these are not in harmony with Oberhänsli's results as cited by Link and Bernauer.[2] It is not possible to draw firm conclusions on this matter before further proof, *e.g.*, chemical correlations, is produced.[14]

The two fluoro pilosine isomers described here should be assigned the same chirality, 2*S*,3*R*,6*R*, since both have negative CD bands around 218 mμ the amplitudes of which significantly compare with that of **3**.

Mass Spectra. The main modes of mass spectral fragmentation for pilosine and related compounds are shown in Scheme II. Examination of mass spectral data assembled in Table III reveals that the ion of highest intensity is the 1-methyl-5-imidazolylmethyl cation (**24**, *m/e* 95), which is accompanied by one or more of the tautomeric forms of 1,5-dimethylimidazolium cation (**25**, *m/e* 96). Other prominent peaks are the molecular ion and the ionic fragments corresponding to M − H_2O, M − H_2O − HCO_2, M − XC_6H_4CHO, M − XC_6H_4CHO − C_2H_2O, M − $C_9H_7O_3$, ionized corresponding benzaldehyde, and benzoylium ion. This suggests that there are five principal electron impact induced fragmentations of the pilosines, comprising three modes of C–C bond ruptures and two of C–O. These involve (a) cleavage of the 2,6 bond with retention of charge by either moiety [*m/e* 180 (**22**) ← M^+· → *m/e* 106 or 124]; (b) cleavage of the 3,5 bond, with or without hydrogen rearrangement, but with retention of the charge on the imidazole substituent (M^+· → **24** and **25**) which could transform into its valence tautomer, *N*-methylpyrimidinium ion; (c) rupture of the lactone ring at two sites, (i) between carbons 2 and 3, and (ii) between carbon 4 and the ring oxygen to yield an ionic species of structure **19**; (d) loss of water involving a hydrogen at C-3, to yield an ion of structure **20** rather than that of **12**, since the ionic distribution in the mass spectra of pilosine stereoisomers (**1**–**4**) is entirely different from that of **12** (see Table III). The 2.8- and 5.6-fold greater abundances of the respective ions **20** and **21** in the mass spectrum of the 2,3-cis (pilosine) as compared to that of the trans isomer

Scheme II



(isopilosine) (see Table III) suggests that the primary reaction $M^{+}\cdot \rightarrow$ **20** could be of considerable diagnostic importance.

Of particular interest, because of its variation with the stereochemistry, is another primary mass spectral reaction featured by the loss of hydrogen from the parent molecular ion (M). Thus, the [M − 1]/[M] ratio in isopilosine is 1.6-fold greater than in pilosine, and 1.4-fold greater than in epiisopilosine. This observation merits further work to establish whether it would be possible to deduce the stereochemistry in the imidazole alkaloids, and in related lactones of the Podophyllum-Lignane series,[10] by mass spectrometry.

Experimental Section

Ir spectra were recorded on a Perkin-Elmer Model 237 spectrometer, uv spectra on a Unicam Model Sp 800A spectrometer, nmr spectra on a 60-MHz Varian spectrometer with TMS as internal standard, optical rotations on a Perkin-Elmer polarimeter, CD spectra with a Cary 60 recording spectropolarimeter, and mass spectra on a Varian MAT CH-5 spectrometer using the direct inlet system. The electron energy was maintained at 70 eV and the ionization current was maintained at 20 μA. The abundances of ions from primary fragmentations are given in percentages relative to the *m/e* 95 ion peak and assembled in Table III. Thin layer chromatographies were performed on silica gel plates.

γ-Crotonolactone, bp 102–107° (24 mm), was prepared according to the literature.[15]

Ethyl γ-phthalimidoacetoacetate (14), mp 111–113° (lit.[16] mp 110°), was obtained in 63% yield by steam-induced hydrolysis of diethyl phthalimidacetomalonate (mp 66–68°, 65% yield) according to Mehrotra and Verma.[16]

Isolation of Pilosine According to Voigtlander and Rosenberg.[5] The title product (recrystallized from ethanol), mp 176–178°, $[\alpha]^{25}D$ +84 ± 1° (*c* 1, EtOH) ("pilosine"), was isolated (0.8–1.3%) from the weak acidic mother liquor left after recrystallization of pilocarpine nitrate which was obtained from the leaves of *Pilocarpus microphyllus* according to the literature.

Separation of "Pilosine" into Pure Forms of Pilosine and Isopilosine. To a suspension of "pilosine" (23 g), $[\alpha]^{20}D$ +82° (*c* 1, EtOH), in 35 ml of water, concentrated nitric acid was added dropwise until pH 3 was reached. While the acid was being added, the suspension gradually turned into a clear solution. After a few crystals of isopilosine nitrate were added and scratching was performed to initiate crystallization, the solution was kept at 0° for more than 2 days. The crystalline isopilosine nitrate thus obtained was collected by filtration (10 g, 35% yield), mp 77–82°. The free base was recovered from the nitrate salt in the usual way: ir (KBr) 1765 cm^{-1}; uv (C_2H_5OH) λ_{max} 214, 249, 253, 259, 265, 269 mμ (ε21,700, 110, 144, 180, 143, 75); CD (C_2H_5OH) λ_{max} 217 ± 1, 261, 267 mμ (θ +15,660 ± 11.5%, −325, −250).

After neutralization of the mother liquor with 10% NaOH and filtration, 14.7 g of a solid was obtained. Recrystallization from 10 volumes of EtOH yielded 9.2 g of crystals, mp 180°, $[\alpha]^{20}D$ +89° (*c* 1, EtOH). The mother liquor was concentrated to ⅓ of the volume and kept at 0° for 2 days. Pilosine dihydrate (2.5 g) crystallized out, mp 101–103°, $[\alpha]^{20}D$ +116.7° (*c* 1 EtOH). Further purification was carried out by recrystallization from absolute EtOH, mp 101–104°, $[\alpha]^{20}D$ +118° (*c* 1, EtOH). By high-vacuum drying of the purified compound while gradually raising the temperature to 105°, anhydrous pilosine was obtained: uv (C_2H_5OH) λ_{max} 212, 249, 254, 259, 265, 269 mμ (ε 15,399, 121, 157, 193, 154, 86); CD (C_2H_5OH) λ_{max} 217, 262, 267 mμ (θ_{max} +21,740, −50, −25); ir (KBr) 1753 and 1763 cm^{-1}.

Isolation of Epiisopilosine from Leaves of *Pilocarpus macrophyllus.* Ground dry leaves (1 kg) of *Pilocarpus jaborandi* were soaked in 800 ml of 6% Na_2CO_3 solution for 1–2 hr. The leaves were then extracted six or seven times, each time with 6 l of benzene at 50–55° for 45 min, until complete extraction of alkaloids was effected. The alkaloids were extracted from the benzene with 5% H_2SO_4, and after decolorization the aqueous layer was gradually basified to pH 9 (5% NH_4OH) while being successively extracted with CH_2Cl_2. The organic extracts were dried on Na_2SO_4 and after evaporation of the solvent 15 g of an oily residue was obtained. To the residue dissolved in acetone (15 ml), dry gaseous HCl was added at a temperature below 15° until a pH of 5.0–5.5 was obtained. The solution was kept at 5° for 24 hr and the crystalline pilocarpine hydrochloride (11–12 g) thus obtained was separated by filtration, washed with acetone, and dried.

The mother liquor was evaporated to yield an oil, which was dissolved in water, decolorized, and neutralized to pH 7.2 with 5% NH_4OH. The aqueous layer was successively extracted with CH_2Cl_2 while the pH was gradually increased to 9. The organic extracts were dried on Na_2SO_4 and the solvent was evaporated. To the residue 1.5 volumes of acetone was added and the suspension thus obtained was kept at 5° for 8–10 hr and then filtered to

yield 0.6–1.0 g epiisopilosine, $[\alpha]_D$ −42° (*c* 1, EtOH). The epiisopilosine was further purified by recrystallization from 7 volumes of dry ethanol: R_f 0.50 (blue spot with iodoplatinic acid) with $CHCl_3$–MeOH (9:1) as eluent, R_f 0.76 with $CHCl_3$–MeOH (7:3); ir (KBr) 1753 cm^{-1}; uv (C_2H_5OH) 212, 249, 254, 259, 262.5, 265.5, 269 mμ (ε 13,600, 114, 149, 181, 156, 142, 106); CD (C_2H_5OH) λ_{max} 218 ± 1, 255, 261.5, 267 mμ (θ_{max} −39,200 ± 9%, +375, +600, +550).

The mother liquor was kept at 5° for an additional 48 hr after a few crystals of isopilosine were added. A mixture of pilosine and isopilosine (1.3–2.0 g) was obtained, $[\alpha]_D$ +45–+65° (*c* 1, EtOH).

Anhydropilosine (12), mp 133–134° (lit.[5] mp 131–132°), $[\alpha]^{25}_D$ +68.8° [lit.[5] $[\alpha]$ +62.3° (*c* 2, EtOH)], ν_{KBr} 1735 cm^{-1} (C=O), uv (C_2H_5OH) 285 mμ (ε 1.8 × 10^4), was prepared by the acetic anhydride induced dehydration of isopilosine according to the literature:[5] CD (C_2H_5OH) λ_{max} 212, 227, 241, 274.5 314.5 mμ (θ_{max} +8840, −6765, +2080, −4720, +2010.

6-Deoxypilosine (18) Nitrate. Anhydropilosine (**12**, 16 g) was dissolved in 180 ml of ethanol, 1 g of 10% Pd/C was added, and the mixture was agitated under atmospheric hydrogen for 2 hr. After removal of catalyst and solvent, the oily residue was dissolved in a 6:1 mixture of methyl ethyl ketone and ethanol, concentrated nitric acid was added to pH 4.5, and the resulting 6-deoxypilosine nitrate (80%) was allowed to recrystallize from dry ethanol: $\nu_{C=O}$ ($CHCl_3$) 1760–1770 cm^{-1}; uv (C_2H_5OH) λ_{max} 233, 249, 254, 260, 263, 266, 269 mμ (ε 1400, 140, 150, 170, 140, 120, 86); CD (C_2H_5OH) λ_{max} 217, 255, 261.5, 268, 272 mμ (θ_{max} +23,280, +550, +990, +1100, −110); mass spectrum *m/e* 270 (M^+), 123, 96, 95 (base peak), 83.

Aldol Cleavage of Epiisopilosine to (3*R*)-Pilosinine (5) and Benzaldehyde. Epiisopilosine (1 g) was dissolved in 40 ml of 20% aqueous potassium hydroxide and allowed to reflux for 8 hr, until benzaldehyde ceased to evolve. After neutralization with dilute sulfuric acid, the precipitating starting material which remained intact was removed, the filtrate was extracted with chloroform, and then solvent was evaporated, leaving behind 780 mg of crystalline (3*R*)-pilosinine (**5**): mp 78–79° (from anhydrous acetic acid); $[\alpha]^{17}_D$ +17.1° (*c* 1, EtOH) [lit.[5] $[\alpha]^{25}_D$ +14.35° (*c* 4, in H_2O)]; ir (KBr) 1758, 1770 cm^{-1}; uv (C_2H_5OH) λ_{max} 224 (sh), 232 mμ (ε 90, 900); CD (C_2H_5OH) 221 mμ, $[\theta]_{max}$ −1485.

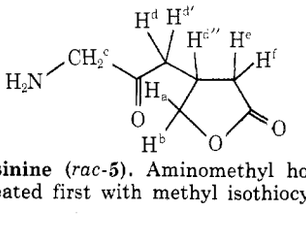
Michael Addition of 14 to γ-Crotonolactone. To a cold solution of sodium ethoxide (17 mmol) in dry ethanol (prepared by dissolving 0.4 g of sodium metal in 40 ml of dry ethanol) was added dropwise with stirring during 8 hr a solution containing a mixture of 10.5 g (125 mmol) of γ-crotonolactone and 13.8 g (50 mmol) of **14** in dry ethanol (40 ml) and the resulting mixture was kept for 24 hr at 50°. Stirring was continued for an additional 24 hr at room temperature, and the resulting precipitate of adduct was filtered, dissolved in benzene, washed with dilute acid, and dried. After the benzene solution was concentrated, the adduct, ethyl α-[3-butyryllactone-γ-(phthalimido)]acetoacetate (**15**), crystallized out (6.1 g): mp 134–135° (from benzene); R_f 0.7 [tlc, MeOH–C_6H_6 (9:1)]; ir ($CHCl_3$) 1783, 1758 (phthalimide), 1728 cm^{-1} (ester).

Anal. Calcd for $C_{18}H_{17}O_7N$: C, 60.16; H, 4.78; N, 3.90. Found: C, 59.90; H, 4.70; N, 3.80.

Further concentration of mother liquor afforded 1 g of the corresponding half-amide (**16**): mp 142–144° (from benzene); R_f 0.2 [tlc, MeOH–C_6H_6 (9:1)]; ir ($CHCl_3$) 1735 (phthalamide), 1725 (ester), 1695 (COOH), 1645 cm^{-1} (internal hydrogen bond).

Anal. Calcd for $C_{18}H_{19}O_8N$: C, 57.30; H, 5.08; N, 3.70. Found: C, 57.10; H, 5.0; N, 3.65.

Acid Hydrolysis of 15 + 16. Formation of Aminomethyl Homopilosinyl Ketone (17). A mixture of **15** (6.1 g) and **16** was dissolved in 20% hydrochloric acid (100 ml) and refluxed for 5 hr, the precipitated phthalic acid was removed by filtration and ether extraction, and the water solution was evaporated to dryness, providing 3.2 g (84%) of the amino ketone hydrochloride **17**: mp 140–143°; ir (KBr) 3420, 1755 (lactone), 1720 cm^{-1} (ketone); nmr (D_2O) τ 5.1–5.3 (1 H, dd, H^a), 5.6–5.9 (1 H, dd, H^b), 5.8 (2 H, s, H^c), 6.9 (3 H, m, H^d + $H^{d'}$ + $H^{d''}$) 7.15 (1 H, dd, H^e), 7.40 (1 H, dd, H^f).



Racemic Pilosinine (*rac*-5). Aminomethyl homopilosinyl ketone (**17**) was treated first with methyl isothiocyanate and then with ferric chloride according to the method of Preobrashensky[7] to yield racemic pilosinine (45%): mp 69–71°; nitrate mp 116–118° (lit.[8] mp 116°); ir (KBr) 1778 cm^{-1} (lactone).

Aldol Condensation between (3*R*)-Pilosinine (5) and Benzaldehyde. The procedure given in the following describes in outline the optimal conditions for the production of pilosine stereoisomers from (3*R*)-pilosinine (**5**) and benzaldehyde. Benzaldehyde (10.6 g, 100 mmol) and (3*R*)-pilosinine (1.8 g, 10 mmol) were mixed and stirred with 60 ml of the appropriate solvent containing 10–40 mmol of the base catalyst, at temperatures and durations of time as indicated in Table II. The reaction mixture was then poured on crushed ice and acidified with hydrochloric acid to pH 1, excess benzaldehyde was removed by extraction with chloroform, and the aqueous phase was first heated for 30 min to accomplish lactonization and then cooled. It was then neutralized to pH 7.2 with the aid of 10% sodium hydroxide, and the resulting precipitate containing reaction products was collected and finally subjected to analysis.

The percentage of anhydropilosine (**12**) was estimated by means of uv spectroscopy, and that of isopilosine and epiisopilosine from both nmr spectroscopy and optical rotations.

Preparation of (−)-Epiisopilosine with the Aid of Lithium Methoxide in Dry *tert*-Butyl Alcohol–Methanol. A mixture of (3*R*)-pilosinine (18 g) and benzaldehyde (106 g) was added with shaking to a solution of lithium methoxide (7.6 g) in a mixture of *tert*-butyl alcohol (400 ml) and methanol (20 ml), allowed to shake for 2 hr at room temperature, and then processed as described above. The reaction product (15 g, $[\alpha]_D$ −23.7°) consisted of 27% isopilosine and 73% epiisopilosine. After two recrystallizations from dry ethanol (7 volumes) the optical rotation of the purified form was found to be constant, $[\alpha]_D$ −44.8° (*c* 1, EtOH).

***o*-Fluoroepiisopilosine (3a) and *p*-Fluoroepiisopilosine (3b).** To a solution of lithium methoxide (2.22 g) in a mixture composed of 150 ml of *tert*-butyl alcohol and 12 ml of methanol was added with stirring (3*R*)-pilosinine (5.4 g, 30 mmol) and *o*-fluorobenzaldehyde (10 g) and the mixture was allowed to shake at room temperature for 24 hr and then processed as described above. Recrystallization from 20 volumes of 95% ethanol yielded 4.3 g of **3a**: ir (KBr) 1757 cm^{-1} (lactone); uv (C_2H_5OH) λ_{max} 211, 256, 261, 267, mμ (ε 7900, 430, 560, 520); CD (C_2H_5OH) λ_{max} 217 ± 1, 245, 252.5, 255, 261, 265, 267 mμ (θ_{max} −34,530 ± 10%, −918, −1570, −1628, −2309, −1598, −1865). From the mother liquor, 2.4 g of a mixture of isomers could be obtained.

Anal. Calcd for $C_{16}H_{17}O_3N_2F$: C, 63.1; H, 5.6; N, 9.2; mol wt, 304. Found: C, 62.6; H, 5.3; N, 9.0; mol wt, 304 (mass spectrum).

In a similar way, epiiso-*p*-fluoropilosine (**3b**) was produced in 47% yield: ir (KBr) 1765 cm^{-1}; uv (C_2H_5OH) λ_{max} 210, 265, 271 mμ (ε 12,500, 800, 740); CD (C_2H_5OH) λ_{max} 218 ± 1, 265, 295 $m\mu_{max}$ (θ_{max} −22,080 ± 6.3%, +190, −266).

Anal. Found for $C_{16}H_{17}O_3N_2F$: C, 62.8; H, 5.3; N, 9.1; mol wt, 304.

Attempted Condensation between 3,4,5-Trimethoxybenzaldehyde and 5. A mixture of (3*R*)-pilosinine (2.7 g, 15 mmol) and 3,4,5-trimethyoxybenzaldehyde (9.8 g, 50 mmol) was added with stirring to a solution of lithium methoxide (1.2 g) in a mixture of *tert*-butyl alcohol (75 ml) and methanol (6 ml) and allowed to stand overnight at room temperature. After processing of the reaction mixture in a fashion described above the starting materials were recovered unchanged.

Registry No.—1, 13640-28-3; **3,** 38993-92-9; **3a,** 51240-45-0; **3b,** 51240-46-1; **4,** 491-88-3; **4** nitrate, 51268-51-0; **5,** 38993-86-1; *rac*-**5,** 38993-85-0; *rac*-**5** nitrate, 51240-47-2; **12,** 51222-06-1; **14,** 13855-80-6; **15,** 51240-48-3; **16,** 51240-49-4; **17** hydrochloride, 51240-50-7; **18** nitrate, 51240-52-9; benzaldehyde, 100-52-7; γ-crotonolactone, 497-23-4; *o*-fluorobenzaldehyde, 446-52-6; *p*-fluorobenzaldehyde, 459-57-4.

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Synthesis of *O*-Methyl-L-serine and N^{α}-*tert*-Butyloxycarbonyl-*O*-methyl-L-serine

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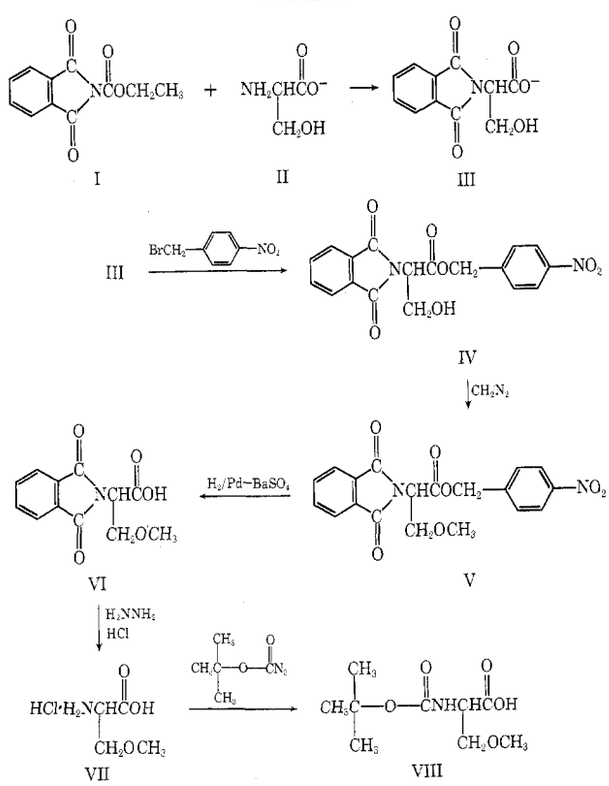
O-Methyl-L-serine and N^{α}-tert-butyloxycarbonyl-*O*-methyl-L-serine were synthesized from L-serine *via* the intermediate N^{α}-phthaloyl-L-serine *p*-nitrobenzyl ester. The ether was made with diazomethane and protecting groups were removed by catalytic hydrogenolysis and hydrazinolysis. Optical purity of the *O*-methyl-L-serine was established. The methyl ether function was stable to the conditions used for acid hydrolysis of peptide bonds and to the acidic conditions used in solid-phase peptide synthesis.

O-Methyl-L-serine was required as an amino acid analog of serine and threonine in the solid-phase peptide synthesis of some new derivatives of ribonuclease A. It was of interest because the hydroxyl groups of serine and threonine can act either as proton donors or acceptors in hydrogen bond formation in proteins, while the methoxyl group of *O*-methyl-L-serine should function only as a proton acceptor. *O*-Methyl-DL-serine was prepared by Schlitz and Carter[3] *via* mercuration of methyl acrylate. Resolution into *O*-methyl-L-serine and N^{α}-acetyl-*O*-methyl-L-serine was achieved by hydrolysis of the N^{α}-acetyl derivative with hog renal acylase.[4] In addition, *N*-phthaloyl-*O*-methyl-L-serine was prepared by Fles and Belenović[5] by resolution of *N*-phthaloyl-*O*-methyl-DL-serine *via* the brucine salt. This paper is concerned with the development of a more convenient synthetic route starting with L-serine.

Four routes were examined: first, a two-step synthesis in which L-serine was acylated to Boc-L-serine followed by methylation of the alcoholic function with methyl iodide in sodium–liquid ammonia at −40°; second, a three-step synthesis in which Boc-L-serine was converted to the *p*-nitrophenyl ester and then methylated with diazomethane (this would have the advantage that the nitrophenyl ester could be used directly for peptide synthesis); third, a four-step synthesis in which Boc-L-serine was converted to the *p*-nitrobenzyl ester with *p*-nitrobenzyl bromide, then methylated with diazomethane, and finally hydrogenated to give Boc-*O*-methyl-L-serine. In preliminary experiments these first three methods were generally unsatisfactory, resulting in poor yields and giving products that were difficult to purify.

The fourth synthesis involved six steps from L-serine but the high yields and easy purification made it the procedure of choice (Scheme I). Phthaloylation of L-serine (II) was carried out in good yield and with retention of optical purity by the method of Nefkens[6,7] using *N*-ethoxycarbonylphthalimide (I) in aqueous sodium carbonate. The *N*-phthaloyl-L-serine (III) was converted to the *p*-nitrobenzyl ester (IV) by a procedure similar to that used by

Scheme I



Shields and Renner[8] for the synthesis of *N*-benzyloxycarbonyl-L-serine *p*-nitrobenzyl ester. IV was methylated under mild conditions with diazomethane in the presence of fluoroboric acid as catalyst[9,10] to give V, which was converted to *N*-phthaloyl-*O*-methyl-L-serine (VI) by catalytic hydrogenolysis. *O*-Methyl-L-serine hydrochloride (VII) was obtained in 53% overall yield from L-serine by hydrazinolysis with hydrazine hydrate and precipitation of the phthalylhydrazide with HCl. Finally, N^{α}-Boc-*O*-