TABLE V

PRINCIPAL NMR OF (3-SUBSTITUTED 2-PIPERIDYL)-2-PROPANONES

	Nmr[a]			
	Cis		Trans	
R	$COCH_3$	CCH_3	$COCH_3$	CCH_3
OCH_3	1.80		1.75	
OC_2H_5	1.82		1.76	
$OCH(CH_3)_2$	1.88		1.82	
CH_3	2.23	0.96	2.11	0.82

[a] 10% toluene solutions except for the 3-methyl compound which was 50% in chloroform. Chemical shifts given in δ (parts per million) from tetramethylsilane.

A 30% solution shows the peaks shifted to δ 1.84 and 1.88 with incomplete resolution. The results were similar in benzene and for the other alkoxy substituents. In the case of the 3-methyl analog, the peaks were at δ 2.11 and 2.23 in $CHCl_3$ for the trans and cis isomers. When toluene is the solvent, care must be taken to avoid interference from the spinning side bands of the solvent methyl group.

Registry No.—**1a**, 39037-79-1; **1a** phenyl isothiocyanate derivative, 39037-80-4; **1b**, 39037-81-5; **1c**, 39037-82-6; **1d**, 39037-83-7; **2a**, 39037-84-8; **2a** phenyl isothiocyanate derivative, 39037-85-9; **2b**, 39004-80-3; **2c**, 39037-86-0; **2d**, 39037-87-1; *cis*-**3**, 39037-92-8; *trans*-**3**, 39037-90-6; **4a**, 39049-96-2; **4b**, 39049-97-3; **4c**, 39049-98-4; **4d**, 39049-99-5; **5a**, 6652-00-2; **5b**, 6651-69-0; **5c**, 39050-02-7; **5d**, 39050-03-8; 3-methoxy-2-picoline, 26395-26-6; acetonitrile, 75-05-8.

The Stereochemistry of Febrifugine. II. Evidence for the Trans Configuration in the Piperidine Ring

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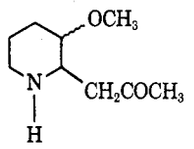
Evidence from nmr spectra and thin layer chromatography is presented pointing to the conclusion that the substituents on the piperidine ring of the hydrangea alkaloid, febrifugine, are in the trans configuration. The absolute stereochemistry proposed is (2′*S*,3′*R*)-3-[3-(3-hydroxy-2-piperidyl)acetonyl]-4(3*H*)-quinazolinone.

The discovery of the facile isomerization of (3-methoxy-2-piperidyl)-2-propanone (**1**),[1] a key intermediate in one synthesis of the hydrangea alkaloid, febrifugine (**2a**),[2] led to a reconsideration of the stereochemistry of the piperidine moiety of febrifugine. Baker, *et al.*, synthesized a second intermediate (**3**)[2]



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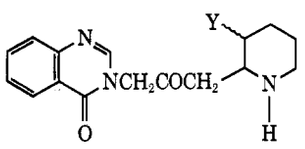
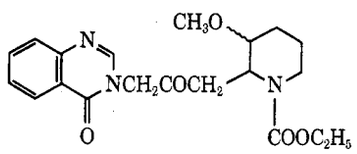


2a, Y = OH, trans
b, Y = OH, cis
c, Y = $OCOCH_3$, trans



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from (3-methoxy-2-piperidyl)-2-propanone (**1**) and showed that it was identical with a sample prepared by two other routes.[3] Compound **3** had previously been converted into febrifugine by removal of the blocking groups and had been assigned the cis configuration (**2b**). However, test results against coccidiosis in chicks with both synthetic isomers of febrifugine and some analogs with substituents on the aromatic ring clearly showed that the trans isomers possessed the expected biological activity. The *trans*-febrifugines are approximately ten times as effective as the *cis*-febrifugines against coccidia in chickens. The activity of the cis isomers is substantially, if not entirely, due to contamination with the trans isomer which is estimated to be present to the extent of 5–10% from thin layer chromatograms.

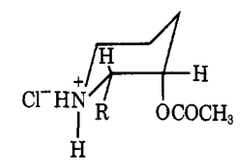
We isolated a sample of febrifugine dihydrochloride from hydrangea according to the procedure of Ablondi, *et al.*[4] The melting point, specific rotation, and ir spectrum all checked with the published data. The free base was prepared according to Hutchings, *et al.*,[5] and its melting point of 156.5–158.5° agreed well with the reported melting point of 154–156°[6] for the higher melting dimorph. The cis and trans racemic febrifugines (**2a,b**) were synthesized by published procedures[1,2] from the isomeric (3-methoxy-2-piperidyl)-2-propanones. Although *trans*-(3-methoxy-2-piperidyl)-2-propanone synthesized by our usual procedure contains from 25–35% of the cis isomer, purification of the febrifugine salts by recrystallization removes the cis isomer and affords the trans compound in high purity.

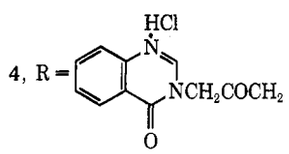
Thin layer chromatography (see Experimental Section) showed that the *trans*-febrifugine was pure, while the cis isomer contained a small amount of the trans. The compound isolated from hydrangea had the same R_f value as the synthesized *trans*-febrifugine. The melting point of the synthesized racemic *trans*-febrifugine, 178.5–180.5°, was higher than that of the naturally occurring dextrorotatory compound. The cis isomer melted at ~134–136°. It was impossible to obtain a precise melting point since the cis isomer underwent a rapid isomerization to the trans compound

(1) D. F. Barringer, Jr., G. Berkelhammer, S. D. Carter, L. Goldman, and A. E. Lanzilotti, *J. Org. Chem.*, **38**, 1933 (1973).
(2) B. R. Baker and F. J. McEvoy, *J. Org. Chem.*, **20**, 136 (1955).
(3) (a) B. R. Baker, R. E. Schaub, F. J. McEvoy, and J. H. Williams, *J. Org. Chem.*, **17**, 132 (1952); (b) B. R. Baker, F. J. McEvoy, R. E. Schaub, J. P. Joseph, and J. H. Williams, *ibid.*, **18**, 153 (1953).
(4) F. Ablondi, S. Gordon, J. Morton, II, and J. H. Williams, *J. Org. Chem.*, **17**, 14 (1952).
(5) B. L. Hutchings, S. Gordon, F. Ablondi, C. F. Wolf, and J. H. Williams, *J. Org. Chem.*, **17**, 19 (1952).
(6) J. B. Koepfli, J. F. Mead, and J. A. Brockman, Jr., *J. Amer. Chem. Soc.*, **71**, 1048 (1949).

near its melting point, and the melting point could be observed only on very rapid heating. The ir spectra of the three compounds were run in arsenic trichloride solution. The spectra of the natural product and the synthesized *trans*-febrifugine were identical while the spectrum of the cis isomer differed in the 900–1250-cm^{-1} region.

The 100-MHz nmr spectra of the diacidic salts of the acetate esters of the natural product and the synthesized *trans*-febrifugine (**2c**) were compared and found to be identical. Acetylation of the cis isomer could not be accomplished; so its nmr spectrum could not be compared with the other two. The resonance of the proton at the point of attachment of the acetonyl side chain in the piperidine ring appeared as a quartet centered at δ 3.98 with a splitting of 7 Hz. This assignment was made by irradiating the sample at the absorption frequency of the side-chain methylene adjacent to the piperidine ring, which collapsed the quartet to a doublet (J = 7 Hz). A coupling constant of 7 Hz for the two methine hydrogens on the piperidine ring is somewhat smaller than would be expected for the trans-diaxial hydrogens in a normal trans disubstituted six-membered ring in the chair conformation, but it is also much too large for the cis isomer. The cis isomer would be expected to prefer the conformation **4** where the acetate ester group is in

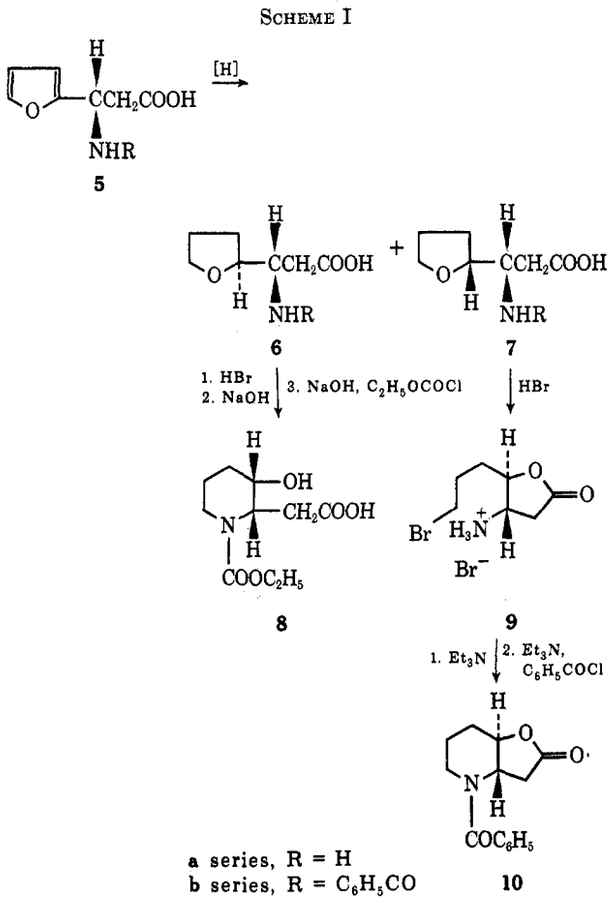




the axial position. In this conformation both methine hydrogens are anti coplanar with electronegative heteroatoms. The axial–equatorial coupling constants observed in similar situations are of the order of 1–2 Hz or less.[7] The resonance for the methine (at 100 MHz) adjacent to the acetate ester function appears as a multiplet centered at δ 5.0. The separation between the outermost lines is about 20 Hz and the pattern seems to fit for coupling constants of about 4–5, 7, and 9–10 Hz. These values indicate that this hydrogen is probably in an axial position.

Since our data all pointed to the conclusion that febrifugine was trans rather than cis as Baker, *et al.*, have proposed,[3b] we decided to examine the earlier work for a possible key to the resolution of this difference. The original stereochemical assignment was made on the basis of the chemistry of intermediates in a synthesis other than the one we used.[3b] This synthesis begins with hydrogenation of a derivative of β-(2-furyl)-β-alanine (**5a**). Baker, *et al.*, reported that, when β-(2-furyl)-β-alanine was hydrogenated over platinum oxide and the crude mixture of tetrahydro compounds (**6a** and **7a**) was treated sequentially with hydrobromic acid at 100°, sodium hydroxide at 100°, and ethyl chloroformate in the presence of sodium hydroxide in the cold, a 1-ethoxycarbonyl-3-hydroxy-2-piperidylacetic acid (**8**) (Scheme I) was obtained which

SCHEME I



was unreactive toward alcohol derivatizing reagents, even after several hours in boiling acetyl chloride. This compound was converted into an isomer of febrifugine and therefore has the "wrong" relative stereochemistry in the piperidine ring.

On the other hand, if *N*-benzoyl-β-(2-furyl)-β-alanine (**5b**) was hydrogenated over palladium on carbon the major product was **7**. When this compound was treated with hydrobromic acid at 100°, the crystalline lactone (**9**) was isolated. This lactone, in the presence of triethylamine in chloroform, cyclized to the lactone of 3-hydroxy-2-piperidylacetic acid. Benzoylation of this amino lactone in the presence of triethylamine yielded the lactone **10**, which was converted into febrifugine.

Baker, *et al.*, concluded that the lactonizable hydroxy acid was cis and the unreactive one trans.[3b] Since our work required the opposite assignment, it was decided to reinvestigate these intermediates. The sequence **5b–7b–9–10** was repeated, and the melting points and chemical properties of all the compounds agreed with those reported.[3b] The ir spectra were in accord with the assigned structures. The nmr spectrum of 1-benzyol-3-hydroxy-2-pyridylacetic acid lactone (**10**) was measured at 60 MHz. The resonance of the proton at C_2 of the piperidine ring appeared as a quartet (J = 8.5 Hz) centered at δ 5.15. The resonance for the C_3 proton appeared as a multiplet centered at δ 4.68

(7) H. Booth, *Tetrahedron Lett.*, 411 (1965); H. Booth and G. C. Gidley, *Tetrahedron*, **21**, 3429 (1965).

which was made up of two triplets (J = 8.5 Hz) separated by 5.0 Hz.

A technique for relating nmr coupling constants to conformation, called dihedral angle estimation by the ratio method (DAERM), has recently been reported.[8] The DAERM method requires a proton coupled to a vicinal methylene group. The dihedral angles are calculated from the ratio (eq 2), of the two Karplus equations (1) using the observed values for J_1 and J_2,

$$J_1 = k_1 \cos^2 \phi_1 - C \qquad 0 \leqslant \phi_1 \leqslant 90° \qquad (1a)$$

$$J_2 = k_2 \cos^2 \phi_2 - C \qquad 90 \leqslant \phi \leqslant 180° \qquad (1b)$$

$$(J_1 + C)/(J_2 + C) = (k_1/k_2)(\cos^2 \phi_1/\cos^2 \phi_2) \qquad (2)$$

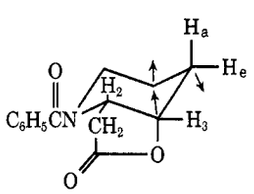
C = 0.28 and k_1/k_2 = 0.9, and substituting $W - \phi_1$ and $W + \phi_1$ for ϕ_2, where W is the H–C–H dihedral angle (usually 120°). The nmr spectrum of 1-benzoyl-3-hydroxy-2-piperidylacetic acid lactone (**10**) reveals that the proton at C_2 of the piperidine ring is coupled to the protons at C_α of the lactone and C_3 of the piperidine ring with J values of 8.5 Hz. The proton at C_3 is in turn coupled to those at C-4 with J values of 5.0 Hz and 8.5 Hz. Using these values and the further condition that at least one dihedral angle (ϕ_1) must be <90° to avoid large strains in the ring, the values in Table I were calculated.

TABLE I

DAERM CALCULATIONS OF 5.0 HZ- AND 8.5 HZ-COUPLINGS

	Obsd coupling constants		Calculated dihedral angles		Calcd Karplus constants	
Set	J_1	J_2	ϕ_1	ϕ_2	k_1	k_2
1	5.0	8.5	40	160	9.00	9.93
2	5.0	8.5	64	56	27.36	28.14
3	8.5	5.0	56	64	28.14	27.36
4	8.5	5.0	15	135	9.41	10.56

Sets 2 and 3 are equivalent and correspond to the cis conformer **11** with an axial oxygen. This structure



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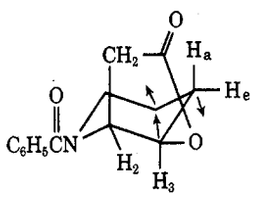

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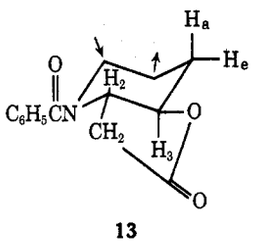

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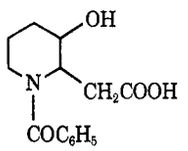
can be eliminated because the Karplus constants are much too large. Sets 1 and 4 have reasonable Karplus constants. The dihedral angles around the C_3–C_4 axis are about the same in the Dreiding models of the cis conformer **12** with the lactone α-methylene in the axial position and in the trans isomer **13**. They are in the range of 40–60 and 160–180°, corresponding quite well with the values calculated in set 1. Using the Karplus equations calculated for set 1, the H–C_2–C_3–H dihedral angle (J = 8.5 Hz) was calculated to be either 9 or 160°. The 160° value compares well with the 160–170° angle shown by the model of the trans isomer, but the angle in the cis conformer **12** is 40°. The angle approaches 0° as **12** is flipped into the boat conformation as indicated by the small arrows. The trans isomer **13** can be flipped into a twist conformer, as indicated by the small arrows, with hardly any change in the dihedral angles of the C_2–C_3–C_4 substituents. Set 4 in Table I requires that the groups on C_3 and C_4 be nearly eclipsed. This does not occur in any of the Drieding models, but it would be the case at some intermediate stage while flipping the conformer **11** into a boat conformer as indicated by the arrows. Thus, while it is not possible to rule out a cis configuration on the basis of the nmr spectrum of 1-benzoyl-3-hydroxy-2-piperidylacetic acid lactone (**10**), the couplings observed seem more easily accommodated by the trans configuration.

It is instructive to compare the reactivity of 1-benzoyl-3-hydroxy-2-piperidylacetic acid (**14**) with the



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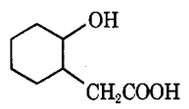

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reactivity of the cis and trans isomers of (2-hydroxycyclohexyl)acetic acid reported by Newman and VanderWerf.[9] The lactone **10** was hydrolyzed with 10% sodium hydroxide. Acidification of the resultant solution with 12 N hydrochloric acid at room temperature precipitated the hydroxy acid **14**. This compound was stable up to its melting point of 157–158°, but was relactonized upon refluxing in acetic anhydride solution for 1 hr. This behavior is similar to that of *trans*-(2-hydroxycyclohexyl)acetic acid (*trans*-**15**), which can be isolated by careful acidification of a cold alkaline solution, but is lactonized by heating to 200° or refluxing several hours in dilute hydrochloric acid solution. The cis isomer of **15**, in contrast, lactonizes spontaneously when an alkaline solution of the salt is neutralized at 0° with the stoichiometric amount of acid. The inertness of (1-ethoxycarbonyl-3-hydroxy-2-piperidyl)acetic acid (**8**) compared to the (2-hydroxycyclohexyl)acetic acids may be due to interaction between the hydroxyl and amide functions. This interaction would be expected to be greater in the cis isomer where the hydroxyl group will be largely in the axial position.

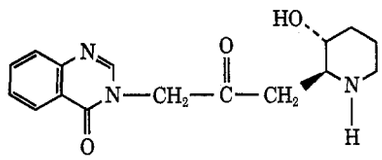
We made some attempts at the resynthesis of *cis*-(1-ethoxycarbonyl)-2-piperidyl)acetic acid (**8**) to compare its spectral and chemical properties with *trans*-(1-benzoyl-3-hydroxy-2-piperidyl)acetic acid. In our hands the hydrogenation of β-(2-furyl)-β-alanine (**5a**) over platinum oxide or 5% rhodium on alumina gave what seemed to be a mixture of the isomeric tetrahydro compounds **6a** and **7a**. However, the subsequent reactions afforded the trans compounds **9** and **10** as the only isolable crystalline products. Thus, we were unable to prepare **8**. Since we did not have pure samples

(8) K. N. Slessor and A. S. Tracey, *Can. J. Chem.*, **49**, 2874 (1971).

(9) M. S. Newman and C. A. VanderWerf, *J. Amer. Chem. Soc.*, **67**, 233 (1945).

of the individual diastereomers **6a** and **7a**, we cannot explain this failure to obtain any **8**.

While some of the chemistry of the intermediates related to febrifugine remains unclear, our results definitely point toward a trans configuration in the piperidine ring. Since Hill and Edwards[10] have determined that the absolute configuration of the 2′ position is *S*, we propose (2′*S*,3′*R*)-3-[3-(3-hydroxy)piperidyl]-acetonyl-4(3*H*)-quinazolinone (**16**) as the structure of



16

febrifugine. The absolute configuration of the hydroxyl group is the same as that in δ-hydroxylysine,[11] which is a plausible precursor, and also the same as 5-hydroxypipecolic acid.[12]

Experimental Section

Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. All melting points are uncorrected. Ir spectra were recorded on a Perkin-Elmer Infracord, Model 137. Nmr spectra were recorded on a Varian A-60 or a Varian HA100.

Febrifugine Dihydrochloride (16 2HCl).—The alkaloid was isolated according to Ablondi, *et al.*, method II.[4] The yield from 1320.65 g of dried hydrangea cuttings (common Easter variety) was 315 mg of the dihydrochloride salt: mp 222–225° dec. (lit.[4] mp 223–225°); ir spectrum (mineral oil mull) identical with the published spectrum;[4] $[\alpha]^{25}D$ +15.3° (standard deviation 3.3°, *c* 0.75, H_2O) {lit.[4] $[\alpha]^{31}D$ +12.8° (*c* 0.85, H_2O)}. *Anal.* Calcd for $C_{16}H_{19}N_3O_3 \cdot 2HCl$: C, 51.3; H, 5.7; N, 11.2; Cl, 19.0. Found: C, 51.5; H, 5.4; N, 11.3; Cl, 19.0.

dl-trans-**Febrifugine Hydrobromide (2a HBr).**—This compound was synthesized from (3-methoxy-2-piperidyl)-2-propanone containing ~70% trans isomer[1] according to a modification of the method of Baker and McEvoy[1,2] using allyl chloroformate rather than ethyl chloroformate to block the secondary amine function. The crude product was recrystallized from 95% ethanol to give the pure trans isomer, mp 229–231°. *Anal.* Calcd for $C_{16}H_{19}N_3O_3 \cdot HBr$: C, 50.3; H, 5.3; N, 11.0; Br, 20.9; OCH_3, 0.0. Found: C, 50.0; H, 5.0; N, 10.9; Br, 20.7; OCH_3, 0.2.

dl-cis-**Febrifugine Dihydrobromide (2b 2HBr).**—This compound was synthesized from *cis*-(3-methoxy-2-piperidyl)-2-propanone using the same sequence as for the trans isomer, mp 199–201°. *Anal.* Calcd for $C_{16}H_{19}N_3O_3 \cdot 2HBr$: C, 41.5; H, 4.6; N, 9.1; Br, 34.5; OCH_3, 0.0. Found: C, 40.5; H, 4.6; N, 8.5; Br, 33.7; OCH_3, 0.3.

Febrifugine Free Bases.—The free base of the natural product was prepared from the dihydrochloride according to the procedure of Hutchings, *et al.*,[5] in 81% yield, mp 156.5–158.5° (lit.[6] mp 154–156° for the higher melting dimorph).

The synthetic stereoisomeric febrifugines were liberated from their salts in the same fashion. The *dl*-trans compound **2a** had mp 178.5–180.5° and the *dl*-cis compound had mp 134–136° (resolidified and remelted at 178.5–181.5°).[13]

Comparison of the spectra in arsenic trichloride solution and the R_f values demonstrated that the naturally occurring compound was the trans isomer. The tlc chromatograms were run by spotting solutions of the alkaloid salts on E. Merck alumina G plates, overspotting with aqueous sodium carbonate, and developing with 5% methanol in benzene. The spots were located by spraying the developed plates with 50% sulfuric acid and heating. R_f values were 0.15 for the trans compounds and 0.3 for the cis.

Febrifugine Acetate Dihydrochloride (2c 2HCl).—This compound was prepared in 43% yield from the natural product according to the procedure of Hutchings, *et al.*:[5] mp 190–194° (lit.[6] mp 184–188°); nmr (100 MHz, D_2O) δ 1.5–2.4 m, 4 H, CH_2CH_2, 2.20 (s, 1 H, CH_3CO), 3.0–3.6 (m, 2 H, NCH_2), 3.43 (d, J = 7 Hz, 2 H, CH_2CO), 3.98 (q, J = 7 Hz, 1 H, NCH), 4.75 (s, exchangeable H), 5.0 (m, 1 H, OCH), 5.26 (s, 2 H, NCH_2CO), 7.65–8.24 (m, 4 H, aromatic), 8.91 (s, 1 H, N=CHN). *Anal.* Calcd for $C_{18}H_{21}N_3O_4 \cdot 2HCl$: C, 51.9; H, 5.6; N, 10.1; Cl, 17.0. Found: C, 52.2; H, 5.6; N, 10.3; Cl, 16.7.

The trans synthetic compound was acetylated in the same manner to give a 71% yield of a diacidic salt, mp 228–229° (from 2B ethanol), which contained 69% chloride and 31% bromide. It was homogeneous by tlc on alumina G (10 and 20% methanol in chloroform and 5 and 20% methanol in benzene). Its nmr spectrum was identical with that of the compound derived from the natural product. *Anal.* Calcd for $C_{18}H_{21}N_3O_4 \cdot 1.38HCl \cdot 0.62HBr$: C, 49.1; H, 5.3; N, 9.6; Br, 9.8; Cl, 11.7. Found: C, 48.7; H, 5.3; N, 8.9; Br, 10.2; Cl, 11.8.

No satisfactory conditions were found for acetylating the *cis* synthetic compound.

1-Benzoyl-3-hydroxy-2-piperidylacetic Acid Lactone (10).—This compound was synthesized according to the procedure of Baker, *et al.*:[3b] mp 99–101° (lit.[3b] mp 99–101°); nmr (60 MHz, $CDCl_3$) δ 1.25–2.60 (m, 4 H, CH_2CH_2), 2.75 (d, J = 8.5 Hz, 2 H, CH_2CO), 3.08 (m, 1 H, axial CH_2N), 3.88 (d of t, 1 H, equatorial CH_2N), 4.69 (m, J = 8.5, 8.5, and 5.0 Hz, 1 H, OCH), 5.15 (q, J = 8.5 Hz, 1 H, NCH), 7.46 (s, 5 H, aromatic).

Registry No.—**2a**, 39037-90-6; **2a** HBr, 39037-91-7; **2b**, 39037-92-8; **2b** 2HBr, 39000-93-6; *trans*-**2c** 2HCl, 39037-93-9; **16** 2HCl, 39037-94-0; *trans*-(3-methoxy-2-piperidyl)-2-propanone, 39037-95-1; *cis*-(3-methoxy-2-piperidyl)-2-propanone, 39037-96-2.

(10) R. K. Hill and A. G. Edwards, *Chem. Ind. (London)*, 858 (1962).
(11) B. Witkop, *Experientia*, **12**, 372 (1956).
(12) B. Witkop and C. M. Foltz, *J. Amer. Chem. Soc.*, **79**, 192 (1957).
(13) The lower melting point is observed only on rapid heating. When the cis isomer is heated slowly, it isomerizes without melting (melting point and mixture melting point).