Tetrahedron Vol. 34, pp. 2421–2431

0040–4020/78/0815–2421/$02.00/0

^{13}C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF ATISINE AND VEATCHINE-TYPE C_{20}-DITERPENOID ALKALOIDS FROM *ACONITUM* AND *GARRYA* SPECIES

NARESH V. MODY and S. WILLIAM PELLETIER*
Contribution from the Institute for Natural Products Research and the Department of Chemistry, University of Georgia, Athens, GA 30602, U.S.A.

(*Received in the USA* 27 *September* 1977; *Received in the UK for publication* 13 *February* 1978)

Abstract—^{13}C NMR spectra of the atisine- and veatchine-type alkaloids, as well as certain of their derivatives, have been obtained by the Fourier transform technique at 25.03 MHz. With the help of single-frequency off-resonance proton decoupling techniques, additivity relationships, and the effects induced by certain structural changes, self-consistent assignments of nearly all the resonances have been made. The ^{13}C NMR spectra are also analyzed to identify skeletal features of the atisine and veatchine-type alkaloids of use in the structure determination of new C_{20}-diterpenoid alkaloids. On the basis of the ^{13}C NMR analysis of atisine and veatchine as well as a temperature-dependence study of atisine, the existence of C-20 epimers in these alkaloids is demonstrated. A ^{13}C NMR study of the behavior of the oxazolidine ring of atisine in non-ionic and ionic solvents indicates that the C-20 epimers of atisine do not exist in an equilibrium mixture in solution and are not interconvertible via a zwitterion as reported earlier.

INTRODUCTION

Recent advances in instruments and techniques have made possible the application of ^{13}C NMR spectroscopy to the study of many naturally occurring substances. In comparison with other techniques, ^{13}C NMR studies are faster, and frequently more reliable than IR, ^{1}H NMR and MS techniques. Moreover, ^{13}C NMR data are also important for the comparison of natural and synthetic compounds or degradation products, especially in the case of the complex diterpenoid alkaloids. ^{13}C NMR spectra may also be used to establish the stereochemistry of compounds which are not available in crystalline form (e.g. atisine). Recently, we have reported the detection of C-20 epimers in atisine **1**, veatchine **17** and related alkaloids by the aid of ^{13}C NMR spectra.[1] We have also examined[2] the unusual behavior of the oxazolidine ring of atisine in ionic and non-ionic solvents by ^{13}C NMR analysis and shown that although atisine exists in solution as a mixture of C-20 epimers, these epimers are neither interconvertible via a zwitterion as reported earlier,[3] nor in equilibrium with each other. The structures of seven new bis-diterpenoid alkaloids have been assigned[4–6] on the basis of ^{13}C NMR analysis. The close structural similarities within the *Aconitum and Garrya* alkaloids[7] suggest that a comparison of the ^{13}C NMR spectra of a series of atisine- and veatchine-type alkaloids and their derivatives will be valuable in confirming or predicting certain stereochemical points as well as assisting structure elucidation and biosynthetic studies of diterpenoid alkaloids. We have, therefore, carried out a ^{13}C NMR study of the atisine and veatchine-type alkaloids with special attention being given to chemical shift data and structural features.

RESULTS

The general procedure for ^{13}C NMR data acquisition and the assignment of the resonances for each of the compounds reported here involved determination of the noise-decoupled spectrum and the single-frequency off-resonance decoupled (SFORD) spectrum. The signals were assigned by means of single-frequency proton off-resonance decoupling techniques,[8,9] application of known chemical shift rules for hydroxyl substitution and acetylation shifts, steric effects and from comparisons of spectra from compound to compound. In addition, deuterated compounds were prepared in some cases and used to confirm the assignment to individual carbons.

Assignments for atisine-type alkaloids

Atisine **1**, *atisinone* **2**, *isoatisine* **3**, *and isoatisinone* **5**.[10,11] Table 1 shows the ^{13}C chemical shifts and assignments for the C-20 epimers of atisine (**1A** and **1B**), and atisinone (**2A** and **2B**) as well as for isoatisine **3** and isoatisinone **5**. In general, the carbons attached to the hetero atom and the methyl- and double-bond containing carbons are easy to assign because they are distinguished by their SFORD spectrum and characteristic chemical shifts. The ^{13}C NMR spectra of atisine (Fig. 1) and atisinone in deuterochloroform at room temperature exhibited two different sets of signals for the oxazolidine ring (ring F), the piperidine ring (ring E), and the C-4 methyl group. The reason for this observation will be discussed later. The downfield chemical shifts at 157.5 ppm (singlet) and at 108.9 ppm (triplet) in atisine **1** are assigned to the double bond between C-16 and C-17. The methine carbon at 77.0 ppm in atisine is assigned to the hydroxyl group at C-15 through the comparison made with the spectrum of atisinone **2**. The low-field doublets at 93.9 and 94.2 ppm are assigned to the C-20 position in both epimers of atisine. These unusual upfield chemical shifts of C-20 in both epimers of atisine, in comparison with values obtained for other oxazolidine ring containing alkaloids, e.g. isoatisine **3**, staphisagnine **6**, and staphisagrine **7** (C-20′ at 100 ppm)[5] can be explained by the steric effect of the C–14—C-8 bond.[12] The three hetero-substituted methylene carbon resonances at 64.1, 56.4 and 50.3 ppm are assigned to C-22, C-19 and C-21,[13] respectively, in the major epimer of atisine **1A**, while the

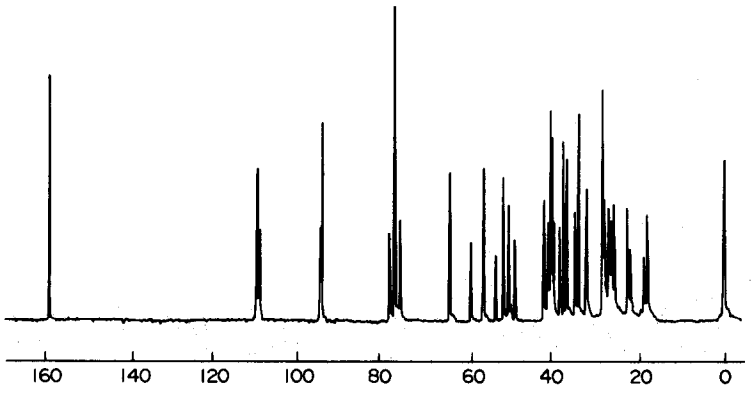


Fig. 1. ^{13}C NMR spectrum of atisine in $CDCl_3$.

Table 1. ^{13}C chemical shifts and assignments for atisine **1**, atisinone **2**, isoatisine **3** and isoatisinone **5**[a]

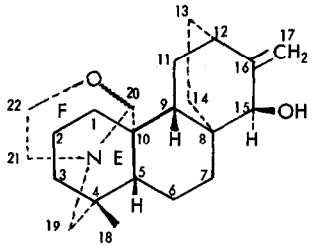
Carbon	1A	1B	2A	2B	3	5
1	42.0[b]	42.0[b]	40.8[b]	40.8[b]	40.6[b]	40.3[b]
2	22.4	21.7	22.4	21.5	22.1	22.0
3	41.0[b]	40.9[b]	40.5[b]	40.5[b]	40.0[b]	39.7[b]
4	33.8	28.2	33.8	27.7	38.1	39.3
5	51.6	48.9	51.5	48.6	48.6	48.3
6	17.8	18.5	17.4	18.2	19.2	18.9
7	34.6	32.0	34.2	34.2	31.9	29.3
8	37.5	37.5	44.7	44.7	37.5	44.4
9	40.0	39.6	44.2	44.2	39.6	43.8
10	40.4	40.4	41.7	41.7	35.9	35.8
11	28.2	28.2	29.6	29.6	28.1	26.2
12	36.6	36.6	36.2	36.2	36.4	35.8
13	27.7	27.7	27.7	27.7	27.6	27.7
14	25.5	25.5	29.3	29.3	26.4	24.9
15	77.0	77.0	204.0	203.0	76.8	202.3
16	157.5	157.5	147.4	147.1	156.2	146.3
17	108.9	108.4	116.3	115.9	109.6	116.7
18	26.7	26.1	26.6	25.9	24.3	24.1
19	56.4	53.3	55.9	53.2	98.4	97.4
20	93.9	94.2	93.4	93.4	49.8	49.2
21	50.3	50.3	50.1	50.1	54.9	54.6
22	64.1	59.2	64.3	59.2	58.6	58.6

[a] Chemical shifts in ppm downfield from TMS. Solvent deuterochloroform.

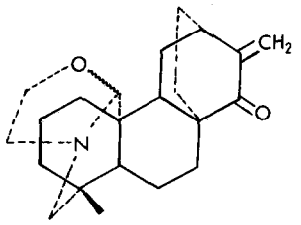
[b] Values in any vertical column may be interchanged.

three triplets at 59.2, 53.3 and 50.3 ppm are assigned to C-22, C-19 and C-21, respectively, in the minor epimer of atisine **1B**. These assignments for C-22, C-19 and C-21, and other chemical shift assignments for the particular epimer of atisine are based on the relative peak height for the specific carbons as shown in Fig. 1. The two methine carbons at 51.6 and 48.9 ppm are assigned to the C-5 position in both epimers of atisine. This assignment for the C-5 position is in good agreement with values found for other diterpenoid alkaloids.[14] The remaining doublets at 40.0 (39.6 ppm) and 36.6 ppm can be assigned to the only available methine carbons at C-9 and C-12, respectively, in atisine.

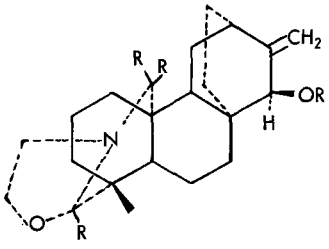
The upfield three quaternary carbon resonances at 33.8 (28.2), 37.5 and 40.4 ppm are assigned unambiguously to C-4, C-8 and C-10; respectively, in atisine **1**. These resonances are assigned on the basis of the observed change in upfield quarternary carbons in atisinone **2** (chemical shift of C-8 moved downfield) and isoatisine **3** (C-4 resonance shifted downfield and C-10 resonance shifted upfield). The only two quartets at 26.7 and 26.1 ppm are assigned to the methyl group present at C-4 in both epimers of atisine. The three methylene resonances at 42.0, 22.4 and 41.0 ppm are assigned to C-1, C-2 and C-3 in ring A, respectively, on the basis of comparisons made with other related diterpenes and their derivatives.[15,16] The remaining chemical shifts in the spectrum of atisine are assigned to C-6, C-7, C-11,



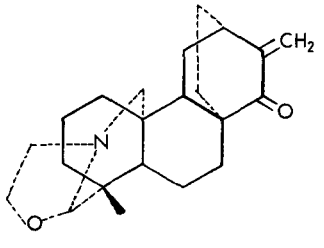
1 Atisine



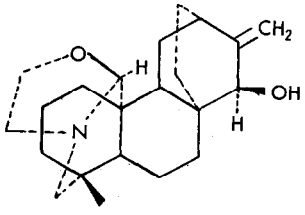
2



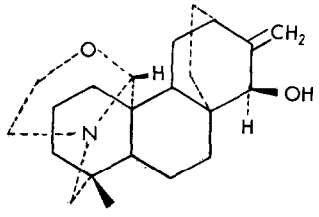
3 R = H Isoatisine
4 R = D



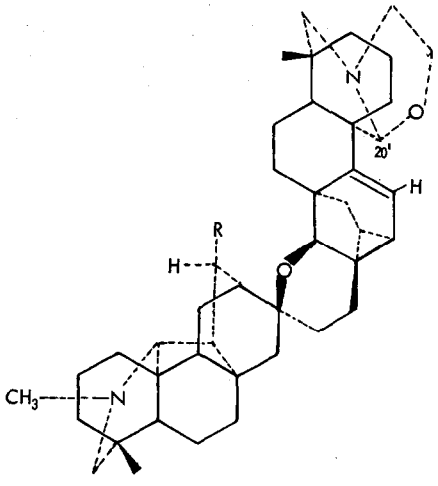
5



1A



1B



6 R = OCH_3 Staphisagnine
7 R = H Staphisagrine

C-13 and C-14. These assignments are based on chemical shift theory and a comparison with the related alkaloids atidine **11** and dihydroatisine **8**, and will be discussed later.

The spectrum of atisinone **2** shows the two different sets of signals for the C-20 epimers as encountered in the spectrum of atisine. When compared with that of atisine, minor changes in the chemical shifts of the double-bond carbons and the presence of a carbonyl group are observed. The downfield chemical shifts at 204.0 and 203.0 ppm are assigned to the ketone group present at C-15 in both C-20 epimers of atisinone. The absence of a doublet at 77.0 ppm is also observed in atisinone **2**, which confirms the assignment of the C-15 hydroxyl group in atisine. At the same time, the downfield shift of C-8 from 37.5 ppm to 44.2 ppm is observed owing to the β-effect of the carbonyl group. As expected the downfield chemical shift of C-16 and the upfield chemical shift of C-17 are observed as shown in Table 1. The change from hydroxyl to the carbonyl group at C-15 moves the chemical shift of C-9 downfield because of the disappearance of a 1,3 diaxial interaction. This observation indicates that the C-15 hydroxyl group and the C-9 proton are in the same conformation in atisine.

The ^{13}C NMR spectrum of isoatisine **3** indicates that this alkaloid does not exist as a pair of C-19 epimers. Because of the change in position of the oxazolidine ring in isoatisine as compared with atisine, some significant changes in the chemical shifts were observed. The methine carbon at 98.4 ppm and the methylene resonance at 49.8 ppm are assigned to C-19 and C-20, respectively (Table 1). These assignments for C-19 and C-20 were confirmed on the basis of the ^{13}C NMR spectrum of 19, 20 deuterated isoatisine **4**.[17] The hetero-substituted methylene resonances at 58.6 ppm and 54.9 ppm can be assigned only to C-22 and C-21, respectively. The downfield shift of C-4 at 38.1 ppm and upfield shift of C-10 at 35.9 ppm in comparison with that of atisine were observed as expected due to the *iso*-position of the oxazolidine ring. The C-4 methyl group was also shifted ~2 ppm upfield owing to the *iso*-type oxazolidine ring in isoatisine.

When the ^{13}C NMR spectrum of isoatisinone **5** is compared with that of isoatisine **3**, some major changes in the chemical shifts of C-8, C-9, C-15, C-16 and C-17 are also observed. These latter changes in chemical shifts of the specific carbons are in agreement with changes observed in the case of atisine **1** and atisinone **2**. All the carbon resonances in isoatisinone were assigned by comparison of the ^{13}C spectrum with those of alkaloids **1**, **2** and **3**. These assignments were also supported by the SFORD spectra.

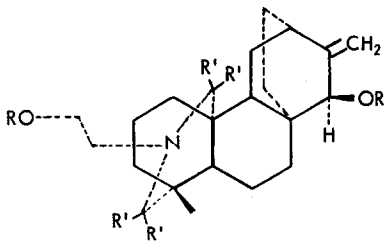
Assignments for dihydroatisine group bases

Dihydroatisine **8**,[10] *dihydroatisine diacetate* **10**,[10] *atidine* **11**,[18] *and atidine diacetate* **12**.[18] The only difference between isoatisine (or atisine) and dihydroatisine is the absence of the oxazolidine ring in dihydroatisine **8**. Comparison of the ^{13}C NMR spectrum of dihydroatisine with that of isoatisine **3**, revealed the absence of chemical shifts due to the oxazolidine ring and the presence of some new chemical shifts due to the $N-CH_2-CH_2-OH$ group. As expected, changes in chemical shifts of C-4 and C-10 relative to isoatisine were evident. The singlets due to C-4 (33.6 ppm) and C-10 (38.0 ppm) were shifted upfield and downfield, respectively in dihydroatisine. The chemical shifts for the other quarternary carbons remain unchanged in **8** as compared with **3**. All the carbons of rings A, C and D in **8** were assigned by comparison of the spectral data with those of the atisine-type alkaloids, 1–5 (Table 1). The hetero-substituted methylene-resonances at 60.2 and 54.0 ppm are assigned to C-19 and C-20 in dihydroatisine, respectively. The chemical shift of C-20 is expected to be further upfield than that of C-19 because of the great steric compression caused by the C-14–C-8 bond. A similar effect was also observed in the case of the chemical shifts of C-19 and C-20 in atisine and isoatisine (Table 1). Further, the assignments of the 19 and 20 carbons were also confirmed by comparison with the spectrum of 19, 20 deuterated dihydroatisine **9**. The remaining hetero-substituted methylene resonances

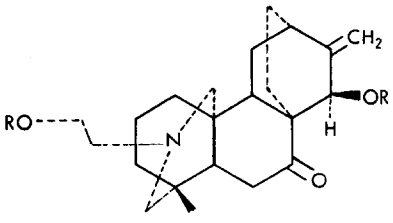
Table 2. ^{13}C chemical shifts and assignments for dihydroatisine-type alkaloids[a]

Carbon	8	10	11	12	13	14	15	16
1	40.2	40.5	40.7	41.0	42.4	42.4	40.6	41.9
2	23.2	23.2	22.6	23.3	20.0	20.0	23.3	22.5
3	41.4	41.8	39.1	39.3	34.1	34.1	31.5	40.7
4	33.6	33.6	33.5	33.5	32.8	32.9	32.4	33.7
5	49.6	49.9	47.9	47.4	46.9	47.0	49.7	49.5
6	17.4	17.3	36.2	36.2	19.6	19.4	17.6	17.4
7	31.5	31.9	215.8	211.5	31.0	31.2	31.6	31.7
8	37.4	36.8	53.0	50.8	37.4	36.7	37.5	37.6
9	39.5	40.5	41.6	42.3	38.1	39.2	39.7	39.6
10	38.0	38.2	37.2	37.3	42.5	42.5	36.5	38.2
11	28.0	28.0	28.0	27.8	28.1	28.0	28.0	28.2
12	36.4	36.4	36.0	36.1	36.0	35.9	35.5	36.5
13	27.7	27.4	26.6	26.8	26.1	25.8	27.7	27.7
14	26.4	26.3	25.3	25.6	25.5	25.0	26.4	26.5
15	76.8	77.2	72.8	73.6	75.2	76.2	76.7	77.0
16	156.3	151.3	151.5	149.2	156.2	151.1	156.4	156.8
17	109.6	110.7	109.5	110.8	108.9	110.1	109.5	109.5
18	26.4	26.3	25.8	25.6	25.8	25.8	26.4	26.4
19	60.2	60.4	58.9	59.1	60.2	60.7	51.8	62.7
20	54.0	53.9	53.5	52.9	166.4	165.1	45.6	56.2
21	58.0	57.2	58.0	57.0	-	-	-	46.9
22	60.7	61.6	60.5	61.1	-	-	-	-
-C=O	-	170.9	-	170.3	-	170.8	-	-
|	-	170.6	-	169.9	-	-	-	-
CH_3	-	21.3	-	21.9	-	21.2	-	-
	-	20.9	-	21.0	-	-	-	-

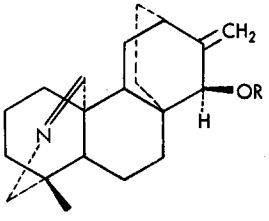
[a]Chemical shifts in ppm downfield from TMS. Solvent deuterochloroform.



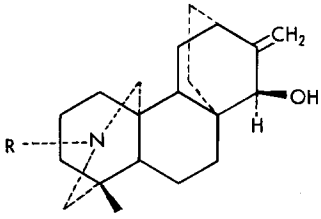
8 R = R' = H
9 R = R' = D
10 R = Ac R' = H



11 R = H
12 R = Ac



13 R = H
14 R = Ac



15 R = H
16 R = CH_3

at 58.0 and 60.7 ppm in compound **8** were assigned to C-21 and a primary hydroxyl group containing carbon at C-22, respectively. On the basis of comparison of the ^{13}C NMR spectrum of **8** with that of atidine **11**, the two upfield triplets in the SFORD spectrum of **8** at 17.4 and 31.5 ppm can be assigned only to C-6 and C-7, respectively.

The ^{13}C NMR spectrum of dihydroatisine diacetate **10** indicated the presence of four more peaks than were present in dihydroatisine. The two carbonyl singlets at 170.6 and 170.9 ppm were assigned to the secondary and primary acetoxyl groups at C-15 and C-22 in **10**. Two quartets at 20.9 and 21.3 ppm for the two acetate methyl groups were also observed. Acetylation of the secondary hydroxyl group at C-15 in compound **10** showed no substitution effect on the chemical shift of C-15, while acetylation of the primary hydroxyl group at C-22 in **10** showed a small substitution effect (~1 ppm downfield). Acetylation of the C-15 hydroxyl group moved the chemical shifts of C-8 and C-16 upfield 0.6 and 5.0 ppm, respectively, in compound **10** relative to **8**. However, the resonances of C-9 and C-17 were shifted downfield 1.0 ppm because of a γ-effect operating in **10**.

The new downfield resonance at 215.8 ppm in atidine **11** is a singlet in the SFORD spectrum and can be assigned to C-7 by comparison with **8**, owing to the presence of a ketone group at that position. The most noticeable observations are the large downfield shifts of C-6 (~19 ppm) and C-8 (~16 ppm) and the small upfield shifts of C-15 (4 ppm) and C-16 (5 ppm) in atidine (relative to **8**) caused by the presence of a ketone group at the C-7 position. The spectrum of an acetylated derivative of atidine **12** indicated the presence of four new peaks due to the C-15 and C-22 acetoxyl groups (Table 2). The substitution of an acetoxyl for a hydroxyl group on C-15 produced an expected ~1 ppm downfield shift relative to compounds **8** and **10**. The neighboring carbons C-8 and C-16 in compound **12** were shifted upfield ~2.2 ppm (β effect) relative to **11**. At the same time, the unusual 3.8 ppm upfield shift (γ-effect) of C-7 is also observed in **12** compared with **11**. The small downfield shifts of C-9 (0.7 ppm) and C-17 (1.3 ppm) due to a γ-effect were also observed in **12**. All other assignments in the ^{13}C NMR spectrum of **12** were confirmed by comparisons made with those of alkaloids **8**, **10** and **11**.

Atisine azomethine **13**, *atisine azomethine acetate* **14**, *dihydroatisine azomethine* **15** *and* N-*methyl dihydroatisine azomethine* **16**.[10] The presence of an imine (–C=N–) group at C-20 in derivative **13** resulted in a number of chemical-shift changes relative to those of compound **8**. The doublet at 166.4 ppm in the SFORD spectrum of **13** is assigned to the C-20 position. Because of the presence of the C-20 imine group in **13**, a downfield (4.2 ppm) shift of C-10 and upfield (0.7 ppm) shift of C-4 were observed relative to **8**. As expected, some changes in the ^{13}C chemical shifts of carbons 1, 2, 3, and 5 of ring A were also observed as shown in Table 2. The resonance for the hydroxyl group at C-15 in **13** is moved 1.6 ppm upfield relative to **8**. Acetylation at C-15 produced a small downfield (~1 ppm) shift in compound **14**. The hetero-substituted methylene resonances at 60.2 ppm in **13** and at 60.7 ppm in **14** are assigned to the C-19 position in both compounds.

Examination of the ^{13}C NMR spectrum of dihydro derivative **15** of atisine azomethine revealed the absence of peaks at 166.4 and 60.2 ppm for the imine and the *N*-substituted methylene groups and the presence of two new peaks for the *N*-substituted methylenes. The triplets at 51.8 and 45.8 ppm were assigned to C-19 and C-20, respectively in compound **15**. The changes in chemical shifts of carbons 1, 2, and 3 of ring A in **15**, as compared with compound **13** were as expected. The introduction of the N–CH_3 group in place of N–H resulted in a number of chemical shift changes in the piperidine ring (ring E)

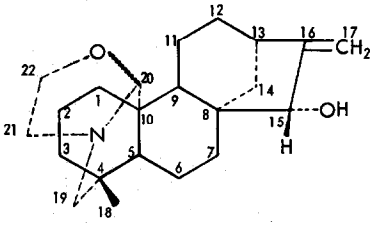
of compound **16**. The resonances for C-19 and C-20 were shifted 10.9 and 10.6 ppm downfield, respectively, in **16** relative to **15**. These changes in chemical shifts of C-19 and C-20 are in agreement with those found in piperidine-containing alkaloids.[19] A quartet at 46.9 ppm in the SFORD spectrum was observed, owing to the presence of the N–CH_3 group in **16**.

Assignments for veatchine-type alkaloids

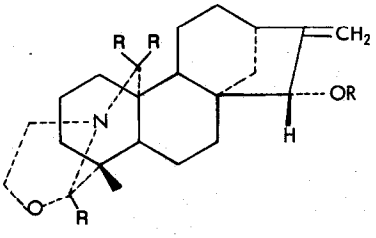
Veatchine **17**[20–23] *and garryine* **18**.[20–23] The ^{13}C NMR spectrum of veatchine in deuterochlorform at room temperature showed two different sets of signals for the oxazolidine ring F, the piperidine ring E, and the C-4 methyl group. A similar ^{13}C NMR spectrum was obtained for atisine, which also contains a normal-type oxazolidine ring. The two sets of ^{13}C signals result from the presence of C-20 epimers, which will be discussed later. Table 3 represents the ^{13}C chemical shifts and assignments for both epimers of veatchine (**17A** and **17B**), for garryine **18** and other related derivatives. The singlets at 160.7 and 161.2 ppm and triplets at 107.4 and 107.8 ppm are assigned to the exocyclic double bond in both epimers **17A** and **17B** of veatchine, respectively. Downfield doublets at 92.6 and 93.3 ppm assignable to C-20 in both epimers of veatchine **17** are also evident. Three heteroatom-substituted triplets at 56.4, 50.2, and 64.3 ppm are assigned[13] to C-19, C-21, and C-22, respectively, in epimer **17A**. The remaining three methylene resonances at 55.9, 49.8, and 58.8 ppm must therefore be assigned to C-19, C-21, and C-22, respectively, in epimer **17B**.

The ^{13}C NMR spectrum of garryine **18** exhibited only one set of signals for the oxazolidine ring carbons, the piperidine ring carbons, and the C-4 methyl group, a fact which indicates that garryine does not exist as a pair of epimers. The downfield methine carbon at 98.2 ppm is assigned to C-19 and is in agreement with the chemical shift of C-19 in isoatisine (**3**). The methylene resonances at 51.1, 54.8 and 58.7 ppm are assigned to C-20, C-21 and C-22, respectively, in **18**. The assignments to C-19 and C-20 in garryine **18** were confirmed by comparison with the spectrum of 19, 20 deuterated garryine **19**.[24] The upfield quarternary carbon resonances at 40.3 and 35.9 ppm can be assigned only to C-4 and C-10, respectively, because of the *iso*-oxazolidine ring in **18**. The remaining carbon resonances in veatchine and garryine were assigned, on the basis of earlier observed ^{13}C chemical shifts in the atisine-type alkaloids and kaurene-type diterpenes.[16]

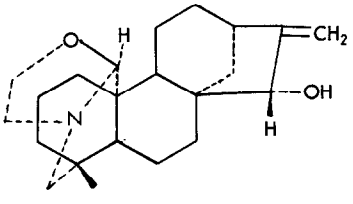
Dihydroveatchine **20**[25] *and dihydroveatchine diacetate* **23**.[25] The ^{13}C NMR spectrum of dihydroveatchine **20** shows no chemical shifts assignable to the oxazolidine ring. Some new ^{13}C chemical shifts were observed in **20** as compared with **18**. The upfield quarternary carbons resonances at 33.6 and 40.2 ppm are assigned to C-4 and C-10, respectively, in dihydroveatchine **20**. These assignments are in good agreement with observed chemical shifts in dihydroatisine **8**. The nitrogen substituted methylene resonances at 60.2, 55.9 and 57.8 ppm in dihydroveatchine can be assigned only to C-19, C-20 and C-21, respectively. The methylene resonance at 60.6 ppm is assigned to the primary hydroxyl group at C-22 in **20**. Also, the methine carbon resonance at 82.3 ppm is assigned to the secondary hydroxyl group at C-15. When the ^{13}C NMR spectrum of diacetate **21** was examined, the presence of some new peaks in the down- and upfield regions of the spectrum of the acetoxyl groups was observed. The introduction of an acetoxyl group at C-15 showed no α-effect on the chemical shift of C-15 in **21** relative to **20**. The only effect of the C-15 acetoxyl was



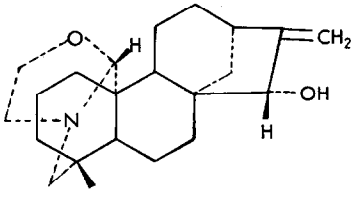
17 Veatchine



18 R = H Garryine
19 R = D



17A



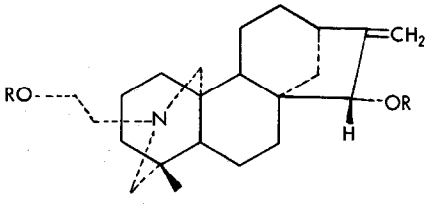
17B

Table 3. ^{13}C chemical shifts and assignments for veatchine-type alkaloids[a]

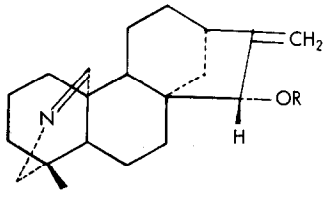
Carbon	17A	17B	18	20	21	22	23	24	26
1	41.7	41.3	40.6	41.2[b]	41.6[b]	42.3	42.4	40.8[b]	41.7[b]
2	18.6	19.2	20.6	18.5	18.3	18.3	18.4	18.3	18.2
3	37.1	37.1	40.6	40.7[b]	40.9[b]	34.9	35.4	40.3[b]	41.2[b]
4	34.1	34.1	40.3	33.6	33.6	32.9	32.9	32.7	33.8
5	52.8	52.3	50.6	50.4	49.9	49.7	49.0	51.0	50.6
6	18.6	17.4	18.2	18.2	18.3	18.3	18.4	18.3	18.2
7	33.9	33.9	33.8	33.2	32.7	32.9	31.8	33.6	33.4
8	47.3	47.5	47.4	47.2	47.0	47.3	47.0	47.5	47.4
9	51.6	51.1	49.1	50.0	49.9	49.7	49.0	50.7	50.0
10	40.6	40.3	35.9	40.2	40.2	45.5	45.5	39.2	40.3
11	22.7	21.8	22.3	23.4	22.4	20.9	20.6	23.7	22.7
12	31.2	30.3	32.4	32.3	32.4	32.9	33.1	32.3	32.4
13	42.4	42.4	41.7	41.7	41.9	42.3	42.2	41.9	41.9
14	35.1	35.1	36.8	36.8	37.6	34.6	34.9	36.5	36.7
15	82.8	84.3	82.7	82.3	82.7	80.6	81.3	82.7	82.8
16	160.7	161.2	159.6	159.1	154.8	159.7	154.8	160.0	159.9
17	107.4	107.8	108.5	108.2	109.9	107.9	109.8	108.3	108.3
18	25.9	26.4	24.4	26.4	26.3	26.0	26.0	26.6	26.5
19	56.4	55.9	98.2	60.2	60.3	58.9	59.5	52.8	62.7
20	92.6	93.3	51.1	55.9	55.8	165.8	165.9	48.0	58.2
21	50.2	49.8	54.8	57.8	57.2	-	-	-	47.0
22	64.3	58.8	58.7	60.6	61.4	-	-	-	-
-C= O	-	-	-	-	170.2	-	170.1	-	-
|	-	-	-	-	170.2	-	-	-	-
CH_3	-	-	-	-	21.0	-	21.0	-	-
	-	-	-	-	21.0	-	-	-	-

[a]Chemical shifts in ppm downfield from TMS. Solvent deuterochloroform.

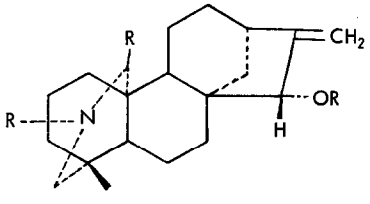
[b]Values in any vertical column may be interchanged.



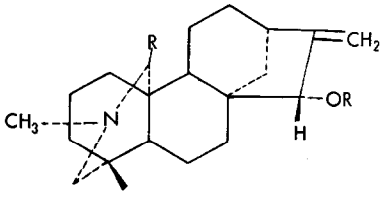
20 R = H
21 R = Ac



22 R = H
23 R = Ac



24 R = H
25 R = D



26 R = H
27 R = D

observed on the chemical shifts of the exocyclic double bond between the C-16 and C-17 positions. The singlet of C-16 shifted 4.3 ppm upfield and the triplet of C-17 moved 1.7 ppm downfield in **21** relative to frequencies in **20**. The replacement of a hydroxyl at C-22 in **20** by an acetoxyl group to give **21** showed very little (0.8 ppm) downfield shift. All the remaining carbon resonances in **20** and **21** were assigned on the basis of comparisons with dihydroatisine **8** and dihydroatisine diacetate **10**.

Veatchine azomethine **22**, *veatchine azomethine acetate* **23**, *dihydroveatchine azomethine* **24** *and* N-*methyl dihydroveatchine azomethine* **26**.[25] The downfield methine carbon resonance at 165.8 ppm is assigned to the imine (–C=N) group at C-20 in **22**. The imine group influences the chemical shifts of carbons in ring A as observed in the case of atisine azomethine **13** compared with derivatives without the imine group.

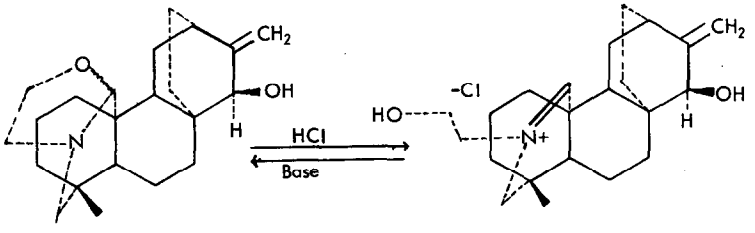
The presence of the imine group at C-20 was confirmed by observing the downfield singlet at 45.5 ppm for C-10 in **22**. The nitrogen substituted methylene resonance at 58.9 ppm can be assigned only to C-19 in **22**. When the imine group of **22** was reduced to the dihydroderivative **24**, the singlet at 45.5 ppm, the doublet at 165.8 ppm and the triplet at 58.9 ppm disappeared and two triplets at 48.0 and 52.8 ppm belonging to C-20 and C-19 were observed in **24**. These assignments in **24** were based on the spectrum of the C-20 deuterated derivative **25**.[26] The downfield singlet of C-10 at 45.5 ppm moved upfield to 39.2 ppm in **24** as compared with that of **22** and **23**. The presence of an acetoxyl group at C-15 in **23** showed a negligible effect on C-15 as observed earlier. When N–H was replaced by the N–CH_3 group in **26**, a downfield quartet appeared at 47.0 ppm. Because of *N*-methylation, the chemical shifts of C-19 and C-20 also shifted ~10 ppm downfield in **26** relative to **24**. The triplet at 58.2 ppm is unambiguously assigned to C-20 by comparison with the spectrum of the C-20 deuterated derivative **27**. Then the remaining nitrogen substituted methylene resonance at 62.7 ppm can be assigned to C-19 in **26**. All other remaining carbons in compounds **22**, **23**, **24** and **26** were assigned as shown in Table 3, on the basis of comparison data, chemical-shift theory and the SFORD spectra as mentioned earlier.

DISCUSSION AND APPLICATION

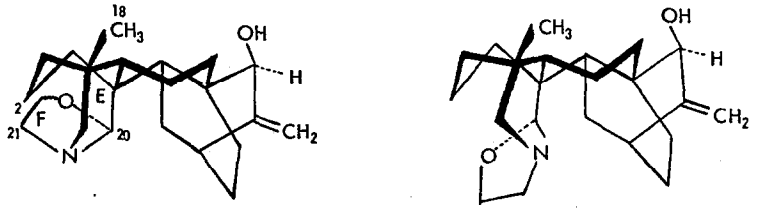
The similar pattern of the ^{13}C chemical shifts exhibited by the series of atisine- and veatchine-type alkaloids and their derivatives made possible the self-consistent and reasonably unambiguous assignment of nearly all carbons in these alkaloids. A particularly important feature is the constant pattern of chemical shifts exhibited by the methylene carbons, except in cases where major structural or substitution changes occur (e.g. at C-7 in atidine **11** compared with **8**). This pattern is valuable in determining the degree of oxygen substitution in these alkaloids, e.g. the ^{13}C spectrum of atidine relative to that of dihydroatisine. The ^{13}C chemical shifts exhibited by the exocyclic double bond and the C-15 hydroxyl group in the atisine- and veatchine-type alkaloids served as a diagnostic test for differentiating the basic skeletons of these alkaloids. In the atisine-type alkaloids, the ^{13}C chemical shift of the C-15 hydroxyl group appears at ~77 ppm, while in the veatchine-type alkaloids, it appears between 82 and 83 ppm. This large difference in the chemical shifts of C-15 is one of several distinctive features useful for identifying the basic skeleton of either type of alkaloid. Large differences were also found between the chemical shifts of the exocyclic double-bonded carbon C-16 in both type of alkaloids providing yet another test for identifying the basic skeletons of these alkaloids. The presence of the normal- or *iso*-type oxazolidine ring in these alkaloids can be determined by observing the presence of doublets at ~93 ppm for the normal-type and at ~98 ppm for the iso-type oxazolidine rings. The ability to assign all three quarternary carbons without ambiguity is useful in detecting substitution occurring in the vicinity of these carbons. The catalogue of ^{13}C chemical shifts of several examples of each type of alkaloid provides a means for the structure determination of new atisine and veatchine-type alkaloids without laborious and time-consuming chemical work.

The conformational analysis of atisine **1**, *veatchine* **17**, *and garryfoline* **30** *and behavior of the oxazolidine ring of atisine*

The structure of atisine, the major alkaloid of *Aconitum heterophyllum*, as **1** was established by workers in



1 28



1C 1D

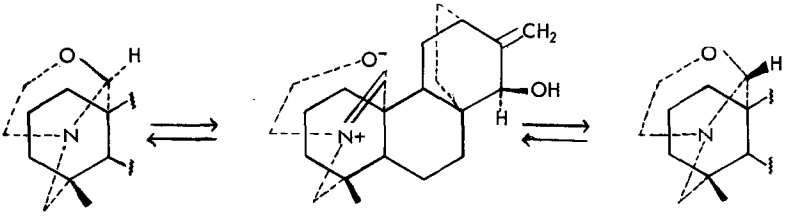
different research laboratories.[10,20,22,27-29] It is amorphous and is usually isolated as the iminium chloride salt **28**, followed by regeneration by treatment with a strong base. Atisine is a very strong base (pK_a 12.8) which is easily isomerized to isoatisine **3** (pK_a 10.3) by refluxing in methanol or other hydroxylic solvents.[11,12]

On the basis of a ^1H NMR study we postulated that atisine exists as two different conformers, **1C** and **1D**, in 1:2 ratio, respectively, in a deuterochloroform solution at room temperature.[30] This postulate seemed to be supported by a temperature-dependence study of the C-4 methyl signals of atisine in benzene. In 1970, Pradhan and Girijavallabhan[3] concluded that atisine in solution contains an equilibrium mixture of the C-20 epimers which are interconvertible via a zwitterion **29**. This conclusion appeared to be supported by a ^1H NMR study of atisine in different polar solvents at 37°C and a deuterium exchange study.[31]

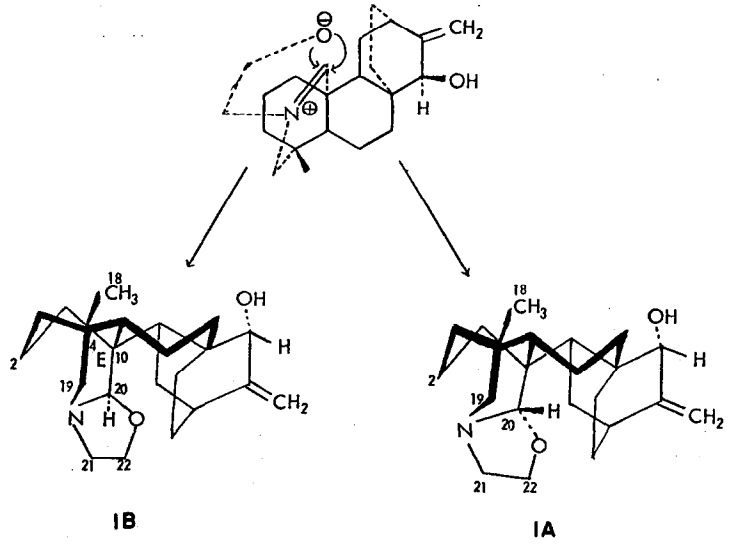
To settle the question of whether atisine is a mixture of conformers **1C** and **1D** or a mixture of C-20 epimers, we must consider the data of Tables 1 and 4. The ^{13}C NMR spectrum of atisine in $CDCl_3$ at room temperature shows two different sets of signals for the oxazolidine ring carbons (ring F), the piperidine ring carbons (ring E), and the C-4 methyl group (Fig. 1). Because rings A and B in the atisine skeleton are held in a rigid conformation, the only conformationally mobile moieties that can give the two sets of signals are the E and F rings. Considering the free energy difference between conformers **1C** and **1D**, two different sets of signals for these two conformers at room temperature are not likely.[32] At room temperature, in solution, the equilibrium between conformers **1C** and **1D** would be so fast that only one set of signals should appear in the ^1H and ^{13}C NMR spectra. Thus we now abandon our earlier hypothesis of a mixture of conformers **1C** and **1D**.

The fact that the oxazolidine ring of atisine is regenerated from atisinium chloride **28** by treatment with base makes possible the formation of the oxazolidine ring from either side of the trigonal C-20 carbon to give two epimers.[33] These C-20 epimers of atisine are represented by structures **1A** and **1B** which are consistent with the ^{13}C NMR spectrum of atisine. The ^{13}C NMR analysis also indicates that epimer **1A** exists in a greater amount than **1B**, presumably because in **1A** the formation of the oxazolidine ring occurs from the less hindered side of the C-20 carbon.

To demonstrate further the existence of C-20 epimers of atisine, a temperature dependence study of atisine in deuterated toluene was performed (Table 4). At 25°C atisine exists as the C-20 epimers **1A** and **1B** in 65% and 35% respectively. At 40°C no significant change in the ratio of these two C-20 epimers in the solution was observed. As the temperature increased progressively to 56° to 70° and to 90°C, new ^{13}C chemical shifts due to the formation of isoatisine appeared, but the two sets of signals for the two epimers did not coalesce to a single resonance. At the same time the ratio of both epimers at different temperatures remained constant. When the spectrum of atisine was taken in toluene, chloroform or in acetone the ratio of eipimers remained constant. A similar behavior was observed also in the case of



1A **29** **1B**



1B **1A**

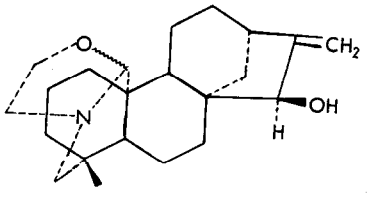
Table 4. A temperature dependence study of atisine[a]

Temp. °C	Atisine 1A[b] %	Atisine 1B %	Isoatisine 2 %
25	65	35	-
40	65	35	-
56	55	35	10
70	55	35	10
90	50	30	20
R.T[c]	50	30	20
R.T[d]	50	30	20

[a]Study was performed in deuterated toluene (d_8) using HMDS [$(CH_3)_3Si]_2O$ as an internal reference. [b]The percentage of compounds in the mixture at a given temperature was determined by monitoring the C-20 peak for **1A** and **1B** and the C-19 peak for 2 in the ^{13}C NMR spectrum. [c]The ^{13}C spectrum was taken at room temperature after the sample had been held at 90°C for 50 min. [d]The spectrum was taken 60 h later at room temp. after temp. had been held at 90°C for 50 min.

atisinone **2**, which cannot serve as a proton source in a non-ionic medium, as is the case with atisine. These observations indicate that the C-20 epimers of atisine do not exist in an equilibrium mixture in non-ionic solvents.

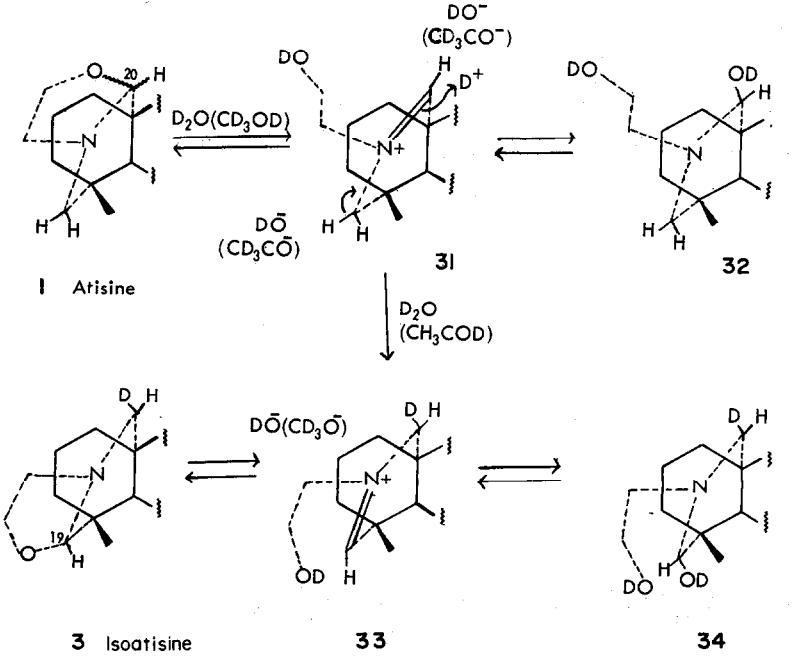
The ^{13}C NMR spectra of atisinone **2** (Table 1) and veatchine **17** (Table 3) also show two sets of signals for the ring E and F carbons, a result which indicates that these compounds also exist as a mixture of C-20 epimers.[1] In the case of veatchine a single crystal X-ray analysis demonstrates the existence of both C-20 epimers in the solid state.[34] Garryfoline[22,35] **30**, a veatchine-type of alkaloid, may also exist as a mixture of C-20 epimers since it is also isolated via the "open" ternary iminium chloride. It should be noted that early work[36] on the absolute configuration of atisine and related alkaloids assumed, without evidence, a β-configuration for the hydrogen at C-20. Because atisine, veatchine, and garryfoline are isolated via the respective ternary iminium chloride, the question concerning which C-20 epimer of each alkaloid occurs in the plant is unanswered.



30 Garryfoline

Our conclusion that atisine and related alkaloids with a normal-type oxazolidine ring do indeed exist as a mixture of the C-20 epimers prompted us to study the behavior of the oxazolidine ring of atisine in non-ionic solvents by the aid of ^{13}C NMR spectroscopy. These results were summarized in earlier communications.[1,2]

When the spectrum of atisine was taken in a solution of 10% D_2O in CD_3COCD_3, isomerization into isoatisine **3** occurred. A spectrum taken using 10% H_2O in CD_3COCD_3 instead of D_2O to see whether the deuterium exchange is taking place at the C-20 position in atisine and the C-19 position in isoatisine showed no difference with the spectrum taken in D_2O. When the ^{13}C NMR spectrum of atisine was taken in CD_3OD, the chemical shifts of carbons 20, 21 and 22 had moved upfield and no new downfield signals for a zwitterion were observed. This result suggests that, in hydrogen-bonding solvents, atisine exists in the form of species **32**, and **34** (via intermediates **31** and **33**) and supports the early postulate of this idea by Wiesner and Edwards.[20] After the ^{13}C spectrum of atisine was taken in CD_3OD (one hour), the solvent was removed and the remaining material dissolved in $CDCl_3$ (free of CD_3OD). The ^{13}C spectrum of the solution now showed the presence of isoatisine as well as the C-20 epimers of atisine. No deuterium exchange at C-20 in atisine and at C-19 in



isoatisine was observed. Similar behavior was also observed in the ^{1}H NMR spectrum of atisine in a solution of 10% D_2O in CD_3COCD_3 as well as in CD_3OD.

The above results lead us to conclude that atisine in non-ionic solvents exists as a mixture of C-20 epimers without interconversion via a zwitterion of any type, and in ionic solvents isomerizes slowly to isoatisine **3** via the zwitterion-type intermediate species **31** and **33**. The Indian workers' interpretation of the broadening of the C-20 proton as indicating epimerization at C-20 and their conclusion that these epimers are interconvertible via a zwitterion are thus in error.

EXPERIMENTAL

^{13}C spectra were determined at 25.03 MHz in the Fourier mode using a JEOL PFT-100 spectrometer in conjunction with an EC-100 20K memory computer. The spectrometer features a deuterium lock system, a JNM-SD-HC random noise (2500 Hz band width) proton decoupler, and JNM-DP-1 digital pulse programmer. Spectra of the compounds were determined in deuterochloroform solutions (which also provided the lock signal) with 5% Me_4Si added as internal reference. All samples were contained in precision ground 10 mm o.d. tubes. The spectrometer was used in the crosscoil configuration. On the average, a 12 μs pulse, corresponding to an approximate tilt angle of 45°, was employed. For the average special width of 5000 Hz or 6250 Hz the delay between pulses was 1 or 2s. Acquisition times averaged 1–2 hours, over 8 K data points for concentrations of the order of 0.4–1.0 M. For off-resonance spectra this time was 4–8 h. The variable high-temperature study was performed in a deuterated toluene (d_8) solution using hexamethyl disilyl ether (HMDS) as an internal standard. The alkaloids and their derivatives used here were purified and/or synthesized by procedures given in the literature cited. Deuterated compounds were prepared as indicated in the references.

Acknowledgements—The authors gratefully acknowledge a matching grant to the Chemistry Department from the National Science Foundation for purchase of the ^{13}C NMR spectrometer. We thank Mr. Courtney Pape for technical assistance in his work and Dr. Wilson De Camp for reading the manuscript and making several helpful suggestions.

REFERENCES

[1]S. W. Pelletier and N. V. Mody, *J. Am. Chem. Soc.* **99**, 284 (1977).
[2]S. W. Pelletier and N. V. Mody, *Tetrahedron Letters* 1477 (1977).
[3]S. K. Pradhan and V. M. Girijavallabhan, *J. Chem. Soc. Chem. Comm.* 644 (1970).
[4]S. W. Pelletier, N. V. Mody, Z. Djarmati, I. V. Mićović and J. K. Thakkar, *Tetrahedron Letters* 1055 (1976).
[5]S. W. Pelletier, Z. Djarmati and N. V. Mody, *Tetrahedron Letters* 1749 (1976).
[6]S. W. Pelletier, N. V. Mody, Z. Djarmati and S. Lajšić, *J. Org. Chem.* **41**, 3042 (1976).
[7]S. W. Pelletier and L. H. Keith, In *The Alkaloids* (Edited by R. H. F. Manske), Vol. 12, Chap. 2. Academic Press, New York (1970).
[8]G. C. Levy and G. L. Nelson, ^{13}C *Nuclear Magnetic Resonances for Organic Chemists*. Wiley-Interscience, New York (1972).
[9]J. B. Stothers, ^{13}C *NMR Spectroscopy*. Academic Press, New York (1972).
[10]S. W. Pelletier and P. C. Parthasarathy, *J. Am. Chem. Soc.* **87**, 777 (1965); and Refs therein.
[11]S. W. Pelletier, R. Aneja and K. W. Gopinath, *Phytochemistry* **7**, 635 (1968); and Refs therein.
[12]S. W. Pelletier, K. Kawazu and K. W. Gopinath, *J. Am. Chem. Soc.* **87**, 5299 (1965).
[13]The earlier reported assignments in Ref. 5 for the chemical shifts of C-21 in atisine and veatchine are in error.
[14]S. W. Pelletier, N. V. Mody, A. J. Jones and M. H. Benn, *Tetrahedron Letters* 3025 (1976).
[15]E. Wenkert and B. L. Buckwalter, *J. Am. Chem. Soc.* **94**, 4367 (1972).
[16]K. Yamasaki, H. Kohda, T. Kobayashi, R. Kasai and O. Tanaka, *Tetrahedron Letters* 1005 (1976); and Refs therein.
[17]19, 20 deuterated isoatisine **4** was prepared by refluxing atisine **1** in CH_3OD under an argon atmosphere on the steam bath for 2 h.
[18]S. W. Pelletier, *J. Am. Chem. Soc.* **87**, 799 (1965).
[19]E. Wenkert, J. S. Bindra, C. J. Chang, D. W. Cochran and F. W. Schnell, *Acc. Chem. Res.* **7**, 46 (1974).
[20]K. Wiesner and J. A. Edwards, *Experientia* **11**, 255 (1955).
[21]K. Wiesner and Z. Valenta, *Progress in the Chemistry of Organic Natural Products*, Vol. XVI, p. 26. Springer-Verlag, Vienna (1958).
[22]H. Vorbrueggen and C. Djerassi, *J. Am. Chem. Soc.* **84**, 2990 (1962).
[23]K. Wiesner, J. R. Armstrong, M. F. Bartlett and J. A. Edwards, *J. Am. Chem. Soc.* **76**, 6068 (1954).
[24]19, 20 deuterated garryine **19** was prepared by refluxing veatchine **17** in CH_3OD under an argon atmosphere on the steam bath for two hours.
[25]S. W. Pelletier and D. M. Locke, *J. Am. Chem. Soc.* **87**, 761 (1965).
[26]The C-20 deuterated derivative **25** was prepared by the reduction of **24** with $NaBD_4$ in CH_3OD at room temperature.
[27]K. Wiesner, R. Armstrong, M. F. Bartlett and J. A. Edwards, *Chem. Indust.* (Lond.) 132 (1954).
[28]S. W. Pelletier and W. A. Jacobs, *J. Am. Chem. Soc.* **76**, 4496 (1954).
[29]J. W. ApSimon and O. E. Edwards, *Can. J. Chem.* **40**, 896 (1962); and Refs therein.
[30]S. W. Pelletier and T. N. Oeltmann, *Tetrahedron* **24**, 2019 (1968).
[31]The reported values for the C-20 protons in Ref. 3 are in error. The C-20 protons in Ref. 3 are in error. The C-20 proton signals of atinine in CD_3COCD_3 or $CDCl_3$ appear between δ 4.0 and δ 4.4 in the ^{1}H NMR spectrum. *Cf.* Ref. 30.
[32]E. L. Eliel, N. L. Allinger, S. J. Angyal and G. A. Morrison, *Conformational Analysis*, Chap. 8. Wiley-Interscience, New York (1965).
[33]W. H. De Camp and S. W. Pelletier, American Crystallographic Association, Evanston, Illinois (Aug. 1976), Abstract 0-7.
[34]W. H. De Camp and S. W. Pelletier, *Science* **198**, No. 4318, 726 (1977).
[35]C. Djerassi, C. R. Smith, A. E. Lippman, S. K. Figdor and J. Herran, *J. Am. Chem. Soc.* **77**, 4801 (1955).
[36]See W. Klyne and J. Buckingham, *Atlas of Stereochemistry. Absolute Configurations of Organic Molecules* Oxford University Press, New York, 1974, p. 171; and Refs therein.
[37]The pattern of ^{13}C NMR spectra of atisine in $CDCl_3$ and CD_3COCD_3 is the same, but the ^{13}C chemical shifts are moved downfield between 1 and 2 ppm in CD_3COCD_3 relative to the shifts observed in $CDCl_3$.