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A 2-D NMR STRUCTURE DETERMINATION OF GUAN-FU BASE Z, A NEW DITERPENE ALKALOID FROM THE CHINESE HERB GUAN-BAI-FU-TZU (ACONITUM KOREANUM)

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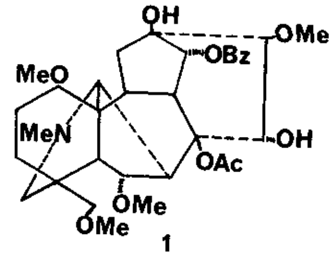
Abstract - By analysis of the ^{1}H-, ^{13}C-, and 2-D-nmr spectra (DEPT, ^{1}J-HETCOR, $^{2,3}J$-HETCOR, HOMCOR) of a new alkaloid, Guan-fu base Z, from Aconitum koreanum its structure was determined to be that of 2-isobutyryl-14-hydroxyhetisine. All the carbon and all but two of the hydrogen resonances could be assigned.

The Chinese herbal drug Guan-Bai-Fu-Tzu (Aconitum koreanum) has been reported to contain several diterpenoid alkaloids of both the C-19[1] and the C-20[2] families. In the former category is hypaconitine **1**[3,4] and possibly a highly-oxygenated base ($C_{35}H_{41}NO_{10}$) from A. koreanum of Russian origin[5], while the latter group includes the so called Guan-fu bases A, B, and G,[3,4,6]. The structure of the last of these has been determined by X-ray crystallography of its methiodide[4,6] to be the triacetoxyaminoalcohol **2**[7]. Guan-fu base A is claimed[9] to be the related diacetoxyaminodiol **3**[7] based on chemical and spectral studies[4a], while Guan-fu base B is a desacetoxy derivative of **3**[3]. Several new alkaloids recently have been isolated from A. koreanum[12], and in this paper the structure of one of them, which we have named Guan-fu base Z, is shown to be 2-isobutyryl-14-hydroxyhetisine (**6**) based primarily on the analysis of its 1-D and 2-D nmr spectra.



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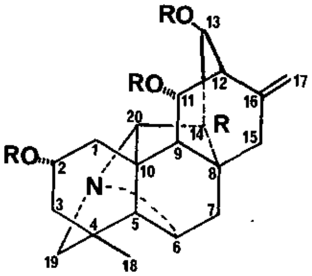


Compound	R^2	R^{11}	R^{13}	R^{14}
2	Ac	Ac	Ac	OH
3	Ac	Ac	H	OH
4	Ac	H	Ac	OH
5	H	H	H	H
6	$COCH(CH_3)_2$	H	H	OH

The utility of ^{13}C-nmr in unravelling the structures of diterpenoid alkaloids with the aid of closely-related model compounds has been convincingly demonstrated by Pelletier et al.[13] Because of the additional information available from high-resolution, 2-D nmr techniques[14-16] it seemed possible that the structure and perhaps even the stereochemistry of Guan-fu Base Z might be determined without the necessity of specific model compounds. This has proven to be the case for $DCCl_3$ solutions on a Varian XL-300 instrument.

The ^{13}C-nmr spectrum of Guan-fu base Z (Table I and Fig. I) consists of 23 lines, the highest field of which was subsequently shown to represent two carbon atoms. The three lowest field peaks were recognized as belonging to an ester carbonyl group (176.51) and an exocyclic methylene group (144.65, 108.17) from their characteristic chemical shifts[13,17]. This was verified from the number of protons on each carbon as determined by a DEPT (distortionless enhancement by polarization transfer) experiment[15,16] as listed in Table I. That the ester is not an acetate is indicated by the absence of the characteristic[17] methyl peak at 20-22 ppm and the acetate carbonyl at 168-171 ppm. An oxazolidine moiety, N-methyl, and N-ethyl groups can also be ruled out since the expected signals[13,17] at 90-99, 40-50, and 11-16 ppm, respectively, are missing.

The ^{1}H-nmr spectrum of Guan-fu base Z (Table II and Fig. I) contains several well-separated, non-exchangeable, single-proton peaks below 3ppm which could

NMR Parameters for Guan-fu Base Z (6)

Table I. ^{13}C

PPM	DEPT	Assignment
176.51	C	1'
144.65	C	16
108.17	CH_2	17
80.23	C	14
79.95	CH	13
76.04	CH	11
69.56	CH	2
69.12	CH	20
63.03	CH	6
62.98	CH_2	19
59.93	CH	5
53.51	CH	9
52.66	CH	12
46.33	C	10
44.30	C	8
37.61	C	4
36.71	CH_2	3
34.41	CH	2'
31.96	CH_2	7
31.37	CH_2	1
31.06	CH_2	15
29.70	CH_3	18
19.08	CH_3	3' (2)

Table II. $^{1}H^{a}$

PPM	Area	Multiplicity (J)	Assignment
5.13	1	m	2
4.86	1	s(br.)	17z
4.68	1	s(br.)	17e
4.22	1	d(8.7)	11
4.04	1	s(br.)	13
3.53	1	s	20
3.11	1	s(br.)	6
2.95	1	d(12.2)	19α
2.85	1	d(15.7)	1α
2.52 [b]	3	d(12.2)	19β
2.50 [b]		sept. (6.8)	2'
2.47 [b]		m	12
2.0 [b]	6		15
2.0 [b]			15
1.98 [b]		m	9
1.86 [b]			1β
1.80 [b]			7α ?
1.77 [b]			3α
1.59	1	dd(15.4,4.1)	3β
1.52	1	s	5
1.37	1	dd(13.9,2.2)	7β ?
1.16	6	d(6.8)	3'
1.01	3	s	18

[a] D_2O exchangable protons omitted; [b] Estimated from 2-D spectra (Tables III-IV, Fig I).

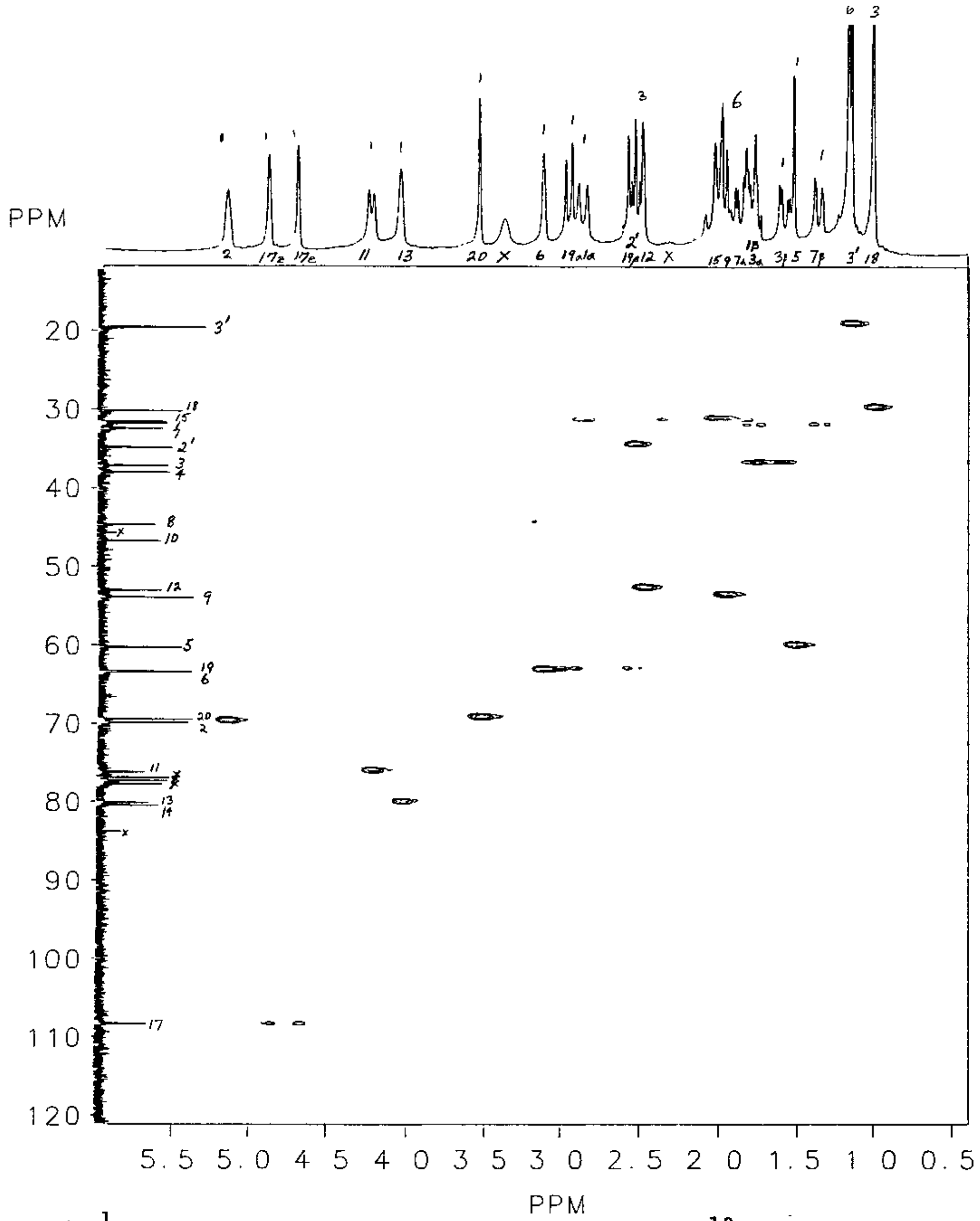


Figure I. ^{1}J-HETCOR experiment of Guan-fu Base Z (6).The ^{13}C spectrum (except for the two lowest-field peaks, cf. Table I) appears on the left and the ^{1}H spectrum with relative areas at the top. Peaks due to solvent, impurities, or exchangeable protons are marked "x".

represent alkene protons and/or hydrogens alpha to oxygen or nitrogen functions. In addition, three sharp three-proton peaks at 1-1.2 ppm suggest the presence of three methyl groups. Since the ^{13}C-nmr shows only two methyl carbon peaks, however, the two peaks centered at 1.16 ppm are probably an isopropyl doublet with J=6.8.

This was verified by a one-bond heteronuclear correlated 2-D nmr experiment (^{1}J-HETCOR)[15,16] (Fig. I) which leads to the connectivities of carbons and their attached hydrogen atoms as assigned in Tables I and II. Thus, all six hydrogens of the 1.16ppm doublet are associated with the single carbon resonance at 19.08ppm thereby establishing that the latter peak does in fact represent two magnetically equivalent methyl groups and that Guan-fu base Z therefore has 24 carbon atoms. Consideration of both the carbon[17] and proton[18] chemical shift values further suggests the presence of 2° oxygen functions at positions 2, 11, and 13, and a 3° one at position 14. Similarly, methine 6 and 20 and methylene 19 appear to be alpha to nitrogen. Although the chemical shifts of carbon 5 and hydrogen 1 are also low enough to be alpha to a nitrogen the values for the attached hydrogen 5 (1.52ppm) and carbon 1 (31.37ppm), respectively, are too high for such an assignment[17,18]. The presence of only one carbonyl group in Guan-fu base Z further indicates that only one of the above oxygen functions is an ester, and that is assigned to position 2 because of the very low-field value of its carbinol hydrogen resonance[19].

From the above results a likely molecular formula for Guan-fu base Z is $C_{24}H_{33}NO_5$ which is verified by the strong molecular ion in the mass spectrum at 415. This leads to an unsaturation number of nine which corresponds to a heptacyclic skeleton plus the ester carbonyl and exocyclic methylene group. Nonadjacent carbon-hydrogen connectivities were obtained directly from a long-range ($^{2,3}J$)HETCOR (Fig. 2 and Table III) (16) and indirectly from a homonuclear correlated 2-D nmr (HOMCOR or COSY) (Fig. 3 and Table IV) experiment[14-16]. Analysis of these connectivities as described in several recent publications[20,21] leads to carbon-carbon connectivities of several structural entities in Guan-fu base Z. For example, the two 3'-methyl groups are attached to the 2'-methine whose proton (2.50ppm) and carbon (34.41ppm) chemical shift are consistent with an adjacent ester carbonyl group[17,18]. Although no isobutyrate esters have been previously reported for diterpenoid alkaloids (1,2) they are known for other terpenoids[22]. The strong peak in the

2-D NMR Correlations of Guan-fu Base Z (6)

Table III. ^{2,3}J-HETCOR

Correlations		Bond
C to	H	Separation
2	1α	2
2	3α	2
3	18	3
4	18	2
5	18	3
5	9	3
6	5	2
6	20	3
8	6	3
8	15	2
8	20	3
10	6	3
10	9	2
10	11	3
11	12	2
11	13	3
12	17e	3
13	11	3
13	12	2
14	9	3
14	12	3
18	19β	3
19	18	3
2'	3'	2

Table IV. HOMCOR

Correlations		Bond	
H to	H	Separation	Comments
1α	1β	2	
1α	2	3	
1α	3α	4	planar "W"
1α	6	5	planar "W"
1β	2	3	
2	3α	3	
2	3β	3	
2	18	5	planar "W", weak
2	19β	5	extended "W", weak
3α	3β	2	
5	6	3	
5	9	4	weak
5	20	4	weak
6	7α	3	
6	7β	3	
6	18	5	very weak
6	20	4	planar W
7α	7β	2	
9	11	3	
9	20	4	weak
11	12	3	
11	13	4	planar W
12	13	3	
15	17e	4	allylic
15	17z	4	allylic
17e	17z	2	
18	19α	4	
19α	19β	2	
2'	3'	3'	

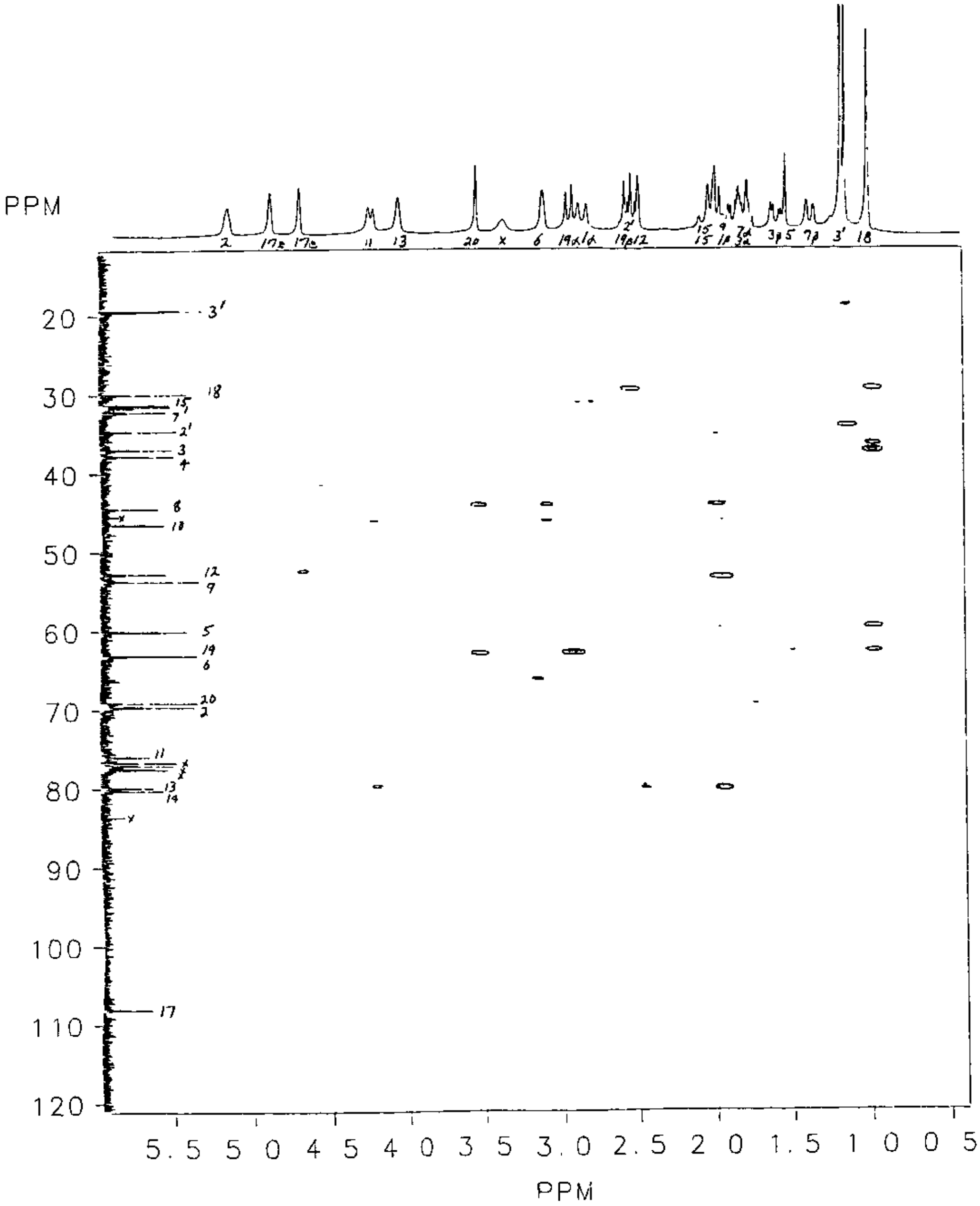


Figure 2. $^{2,3}J$-HETCOR experiment of Guan-fu base Z (6). Caption to Figure 1 applies Some residual ^{1}J-correlations are incompletely nulled.

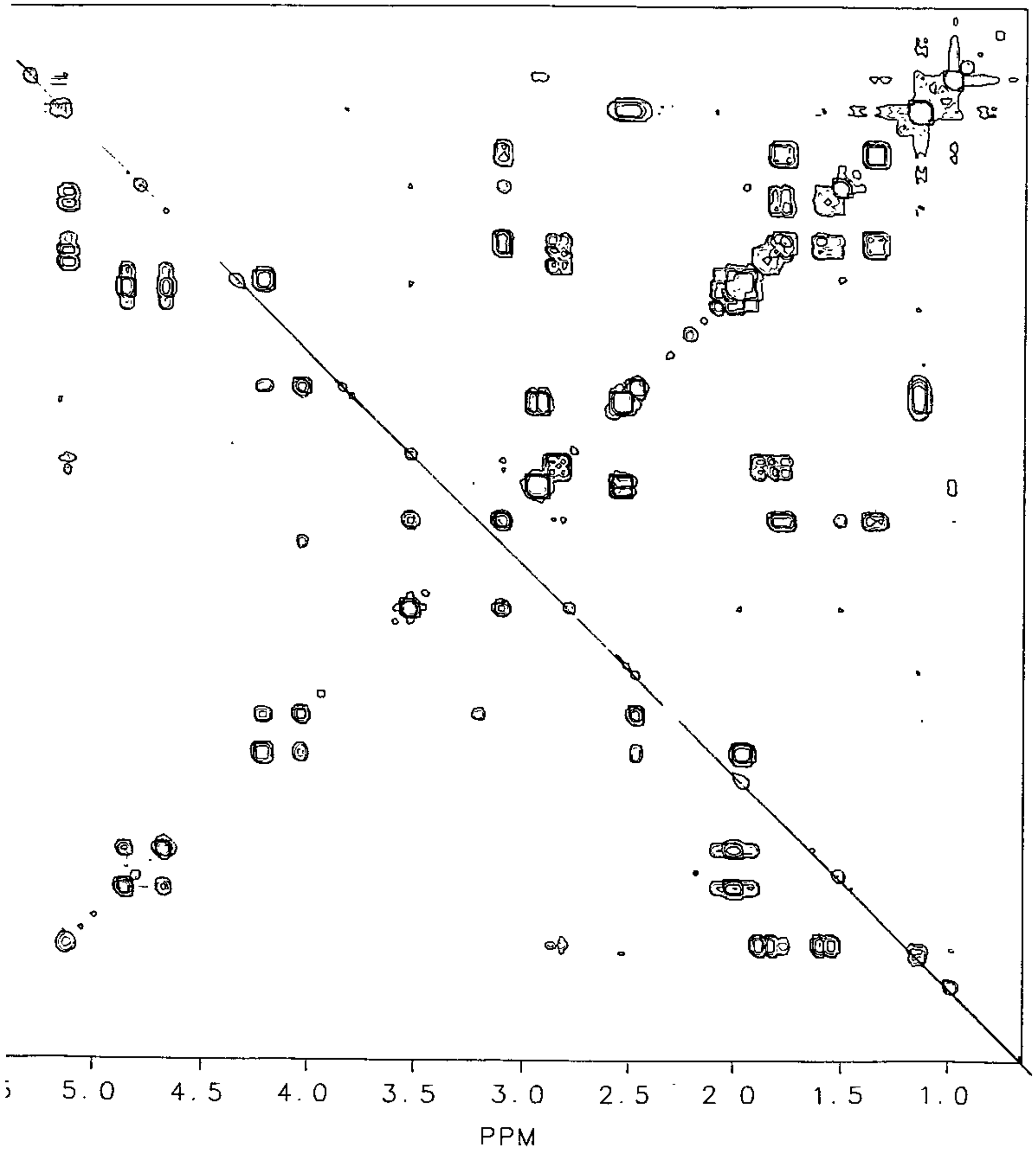
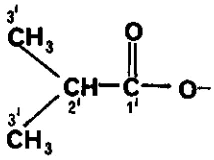


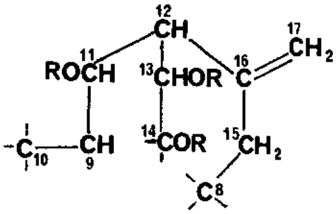
Figure 3. HOMCOR (COSY) experiment of Guan-fu base Z (6). The ^1H-spectrum appears along the lower-left/upper-right diagonal while the lower-right/upper left diagonal may contain artifacts.

mass spectrum at m/x 328(83)(M-87) further confirms that Guan-fu Base Z contains an isobutyrate group A.

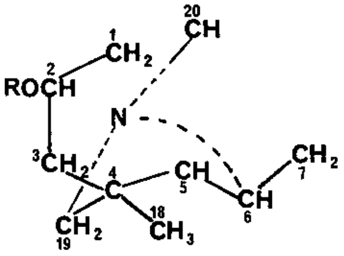
Similarily, the 16,17-alkene is correlated with the allylic 15-methylene and the 12-methine. The latter is associated with the 2^o-oxygen functionalities at 11 and 13 and the 3^o one at 14, while the former is near the quaternary center at 8. The 3^o-oxygen functionality at C-14 is attached to C-13 rather than C-11 because the hydrogen of the latter, but not the former, displays a strong vicinal coupling J_{HH}=8.7 to a methinyl group. This latter group is identified as the one at position 9 from the HOMCOR experiment (Table IV). Finally, the correlation of the quaternary center 10 with both positions 9 and 11 leads to substructure B. Additional relations of C-9 with positions 5, 14, and 20 and C-8 with positions 6 and 20 are also observed but cannot be unambiguously assigned at this point.



A

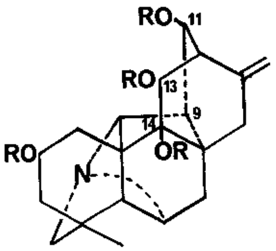


B



C

A third structural feature of Guan-fu base Z is revealed by analysis of the correlations in the vicinity of the sharp singlet 18-methyl resonance. The absence of any vicinal splitting (width at half-height = 2.7 Hz) indicates attachment to one of the quaternary carbons which from the long-range HETCOR must be C-4. The remaining correlations are to positions 3,5, and 19. The last of these is attached to the nitrogen along with methinyls 6 and 20 as noted previously. Position 3 is strongly correlated to the 2^o oxygen function at 2 and the methylene at 1, while position 5 is associated strongly with C-6. The latter also correlates with methylene-7 and methinyl-20 to give substructure C. Taken together B and C account for all of the skeletal carbon atoms of Guan-fu base Z and, along with the heptacyclic skeleton and the absence of N-alkyl or oxazolidine moieties deduced earlier, suggest a structure of the hetisine (5) type. Cross-correlations of B and C at 5-9, 6-8, 6-10, 8-20, and 9-20 support this hypothesis and lead to structure 6 with the stereochemistry at 2, 11, and 13 unspecified for the time being. An alternative connection of B and C to give structure 7 would place the tertiary oxygen function as it is found in the alkaloid sadosine[23]. Such a variation would require interchanging the assignments of positions 11 and 13 as well as of 9 and 14 as shown in 7. This change in turn would result in the long-range HETCOR correlations for C^5-H^9, C^{10}-H^{11}, and C^{10}-H^9 involving separations of 5,4, and 3 bonds rather than the 3,3, and 2 of structure 6 (Table III) and was therefore rejected. The same critera of minimizing the bond separations of correlating atoms was used to distinguish the assignments of methylenes 1 and 7 and methinyls 6 and 20. Similarly, correlations which were ambiguous due to overlapping resonances (i.e. H-15 and H-9 or H-1β, H-3α, and H-7α) were assigned so as to give the smallest bond separations in the HOMCOR or HETCOR experiments.



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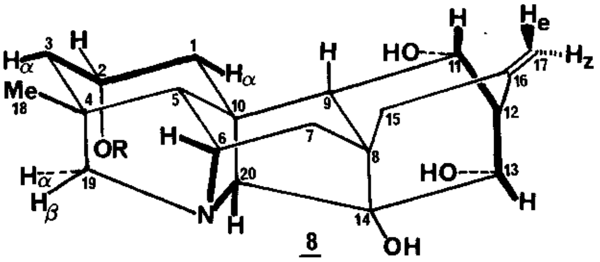

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The alpha-configuration (axial) of the isobutyrate ester group at position 2 was indicated by the fact that the width at half height of H-2 is below 12Hz as would be expected[24] for an equatorial H. The remaining stereochemical features to be determined are the configurations of the secondary alcohols at C-11 and C-13. The former is clearly alpha[8] as shown in 6 and 8 since the magnitude of $J_{9,11}$ = 8.7 implies an <u>endo-endo</u> relation of H-9 and H-11[25]. The 11-OH is similarly assigned on the assumption that the strong $^4J_{11,13}$ - correlation (Table IV) indicates a planar "W" arrangement between H-11 and H-13. In support of this hypothesis the only other <u>strong</u>, nonallylic, ^{4}J-HOMCOR's are for the planar "W"'s H-6 to H-20 and H-1α to H-3α as indicated in 8[26]. This last correlation is also consistent with the assignment of the lower field proton of methylene groups 1, and 3 to the alpha configuration based on the expected[27] deshielding of equatorial over axial protons. The unusually low field resonance for H-1 presumably is due to the proximity of OH-13 and especially OH-11[27]. Similar deshielding by OH-14 more tentatively suggests that the low field proton of methylene 7 has the alpha-configuration.

The nonequivalent methylene protons of 6 at positions 17 and 19 were not assigned directly but, by analogy to the spectra of a closely related alkaloid where appropriate NOE experiments were performed[11], H-19αand H-17z are probably the low field members of these pairs. The protons at position 15 are not sufficiently resolved even at 300 MHz for first order analysis and/or NOE experiments and must therefore remain unassigned at present.

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G is represented with a beta C-13 acetate (and no 14-OH) in reference 6 and in two of three drawings in reference 4a. The projection drawings of the methiodide in references 4a and 4c show the correct alpha stereochemistry for the oxygen functions at C-11 and C-13 as is also found in the related alkaloid hetisine 5[10].

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