

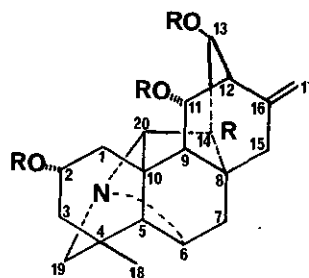
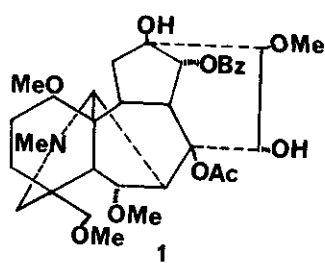
A 2-D NMR STRUCTURE DETERMINATION OF GUAN-FU BASE Z,
A NEW DITERPENE ALKALOID FROM THE CHINESE HERB
GUAN-BAI-FU-TZU (ACONITUM KOREANUM)

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Abstract - By analysis of the ^1H -, ^{13}C -, and 2-D-nmr spectra (DEPT, ^1J -HETCOR, $^2,^3\text{J}$ -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-19¹ and the C-20² families. In the former category is hypaconitine 1^{3,4} and possibly a highly-oxygenated base ($\text{C}_{35}\text{H}_{41}\text{NO}_{10}$) from A. koreanum of Russian origin⁵, 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 triacetoxyminoalcohol 2⁷. Guan-fu base A is claimed⁹ to be the related diacetoxyminiol 3⁷ based on chemical and spectral studies^{4a}, while Guan-fu base B is a desacetoxy derivative of 3³. Several new alkaloids recently have been isolated from A. koreanum¹², 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.



Compound	R ²	R ¹¹	R ¹³	R ¹⁴
<u>2</u>	Ac	Ac	Ac	OH
<u>3</u>	Ac	Ac	H	OH
<u>4</u>	Ac	H	Ac	OH
<u>5</u>	H	H	H	H
<u>6</u>	COCH(CH ₃) ₂	H	H	OH

The utility of ¹³C-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 techniques¹⁴⁻¹⁶ 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 DCCl₃ solutions on a Varian XL-300 instrument.

The ¹³C-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 characteristic¹⁷ 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 ¹H-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			Table II. $^1\text{H}^a$			
PPM	DEPT	Assignment	PPM	Area	Multiplicity (J)	Assignment
176.51	C	1'	5.13	1	m	2
144.65	C	16	4.86	1	s(br.)	17z
108.17	CH_2	17	4.68	1	s(br.)	17e
80.23	C	14	4.22	1	d(8.7)	11
79.95	CH	13	4.04	1	s(br.)	13
76.04	CH	11	3.53	1	s	20
69.56	CH	2	3.11	1	s(br.)	6
69.12	CH	20	2.95	1	d(12.2)	19 α
63.03	CH	6	2.85	1	d(15.7)	1 α
62.98	CH_2	19	2.52 b	3	d(12.2)	19 β
59.93	CH	5	2.50 b		sept. (6.8)	2'
53.51	CH	9	2.47 b		m	12
52.66	CH	12	2.0 b	6		15
46.33	C	10	2.0 b			15
44.30	C	8	1.98 b		m	9
37.61	C	4	1.86 b			1 β
36.71	CH_2	3	1.80 b			7 α ?
34.41	CH	2'	1.77 b			3 α
31.96	CH_2	7	1.59	1	dd(15.4,4.1)	3 β
31.37	CH_2	1	1.52	1	s	5
31.06	CH_2	15	1.37	1	dd(13.9,2.2)	7 β ?
29.70	CH_3	18	1.16	6	d(6.8)	3'
19.08	CH_3	3' (2)	1.01	3	s	18

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

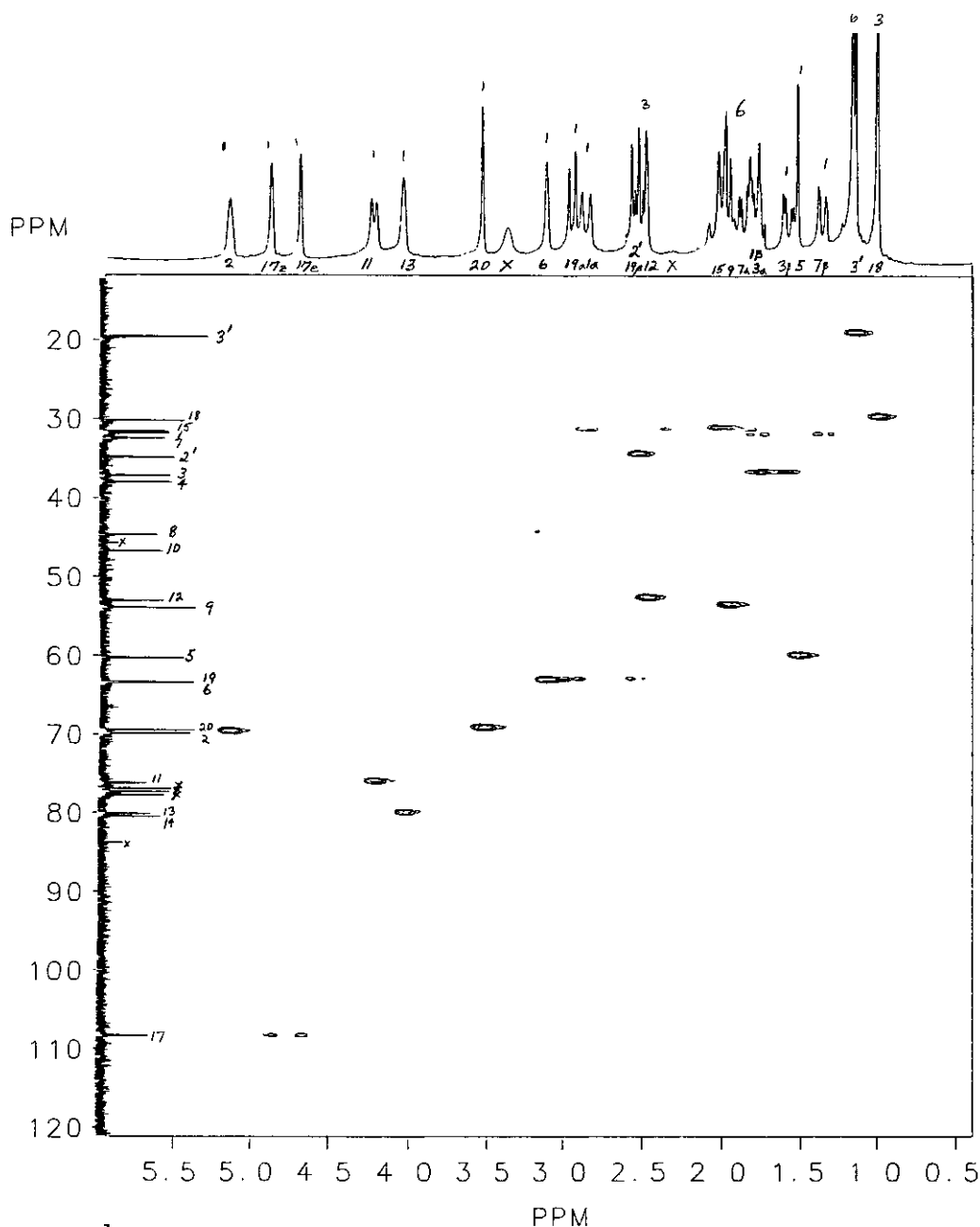


Figure I. ^1J -HETCOR experiment of Guan-fu Base Z (6). The ^{13}C spectrum (except for the two lowest-field peaks, cf. Table I) appears on the left and the ^1H spectrum with relative areas at the top. Peaks due to solvent, impurities, or exchangeable protons are marked "x".

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 (^1J -HETCOR)^{15,16} (Fig. 1) 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 carbon¹⁷ and proton¹⁸ 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 resonance¹⁹.

From the above results a likely molecular formula for Guan-fu base Z is $\text{C}_{24}\text{H}_{33}\text{NO}_5$ 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 ($^2,^3\text{J}$)HETCOR (Fig. 2 and Table III) (16) and indirectly from a homonuclear correlated 2-D nmr (HOMCOR or COSY) (Fig. 3 and Table IV) experiment¹⁴⁻¹⁶. 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 terpenoids²². The strong peak in the

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

Table III. $^2,^3J$ -HETCOR			Table IV. HOMCOR			
Correlations C to H		Bond Separation	Correlations H to H		Bond Separation	Comments
2	1 α	2	1 α	1 β	2	
2	3 α	2	1 α	2	3	
3	18	3	1 α	3 α	4	planar "W"
4	18	2	1 α	6	5	planar "W"
5	18	3	1 β	2	3	
5	9	3	2	3 α	3	
6	5	2	2	3 β	3	
6	20	3	2	18	5	planar "W", weak
8	6	3	2	19 β	5	extended "W", weak
8	15	2	3 α	3 β	2	
8	20	3	5	6	3	
10	6	3	5	9	4	weak
10	9	2	5	20	4	weak
10	11	3	6	7 α	3	
11	12	2	6	7 β	3	
11	13	3	6	18	5	very weak
12	17e	3	6	20	4	planar W
13	11	3	7 α	7 β	2	
13	12	2	9	11	3	
14	9	3	9	20	4	weak
14	12	3	11	12	3	
18	19 β	3	11	13	4	planar W
19	18	3	12	13	3	
2'	3'	2	15	17e	4	allylic
			15	17z	4	allylic
			17e	17z	2	
			18	19 α	4	
			19 α	19 β	2	
			2'	3'	3'	

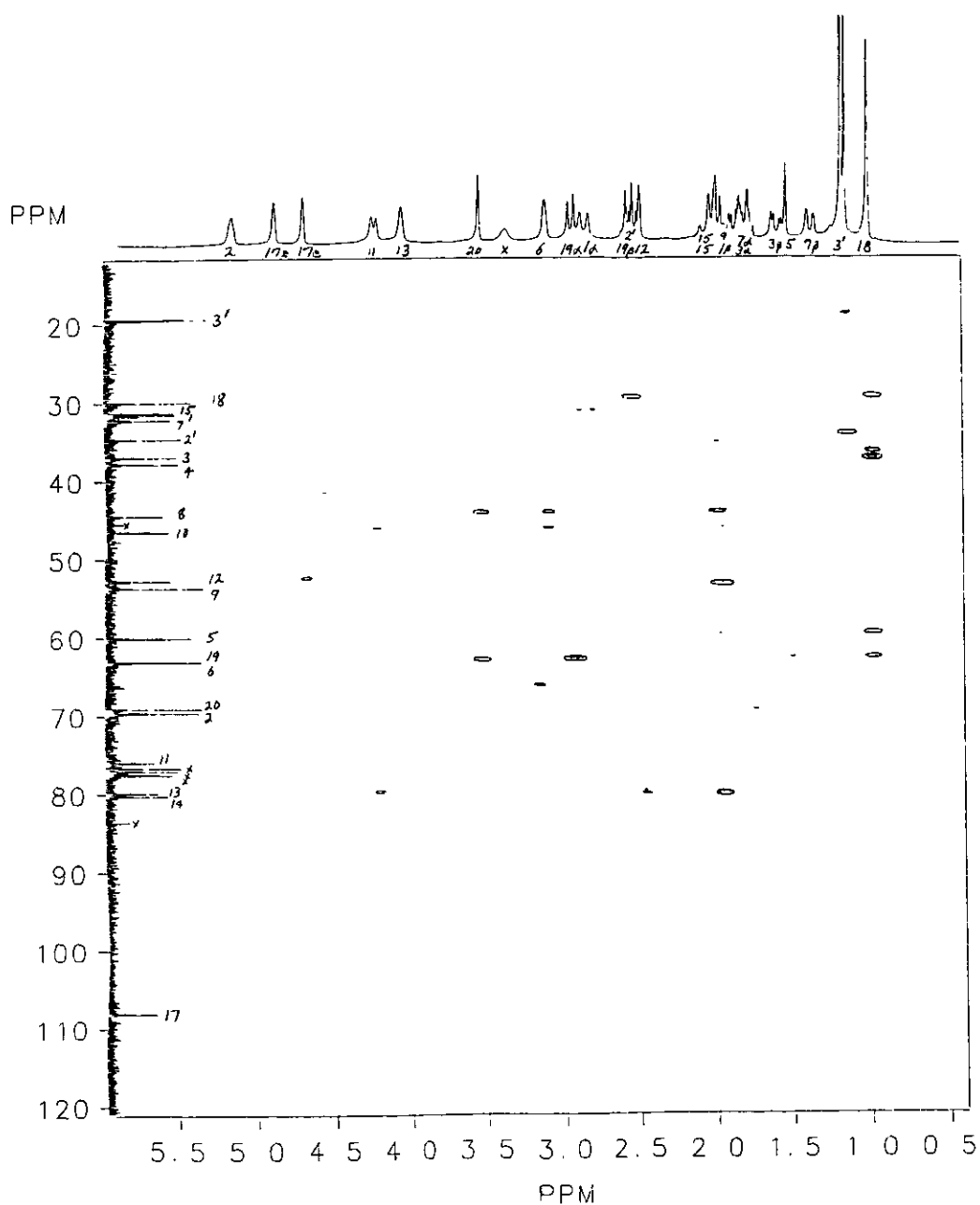


Figure 2. $^2,^3J$ -HETCOR experiment of Guan-fu base Z (**6**). Caption to Figure 1 applies. Some residual 1J -correlations are incompletely nulled.

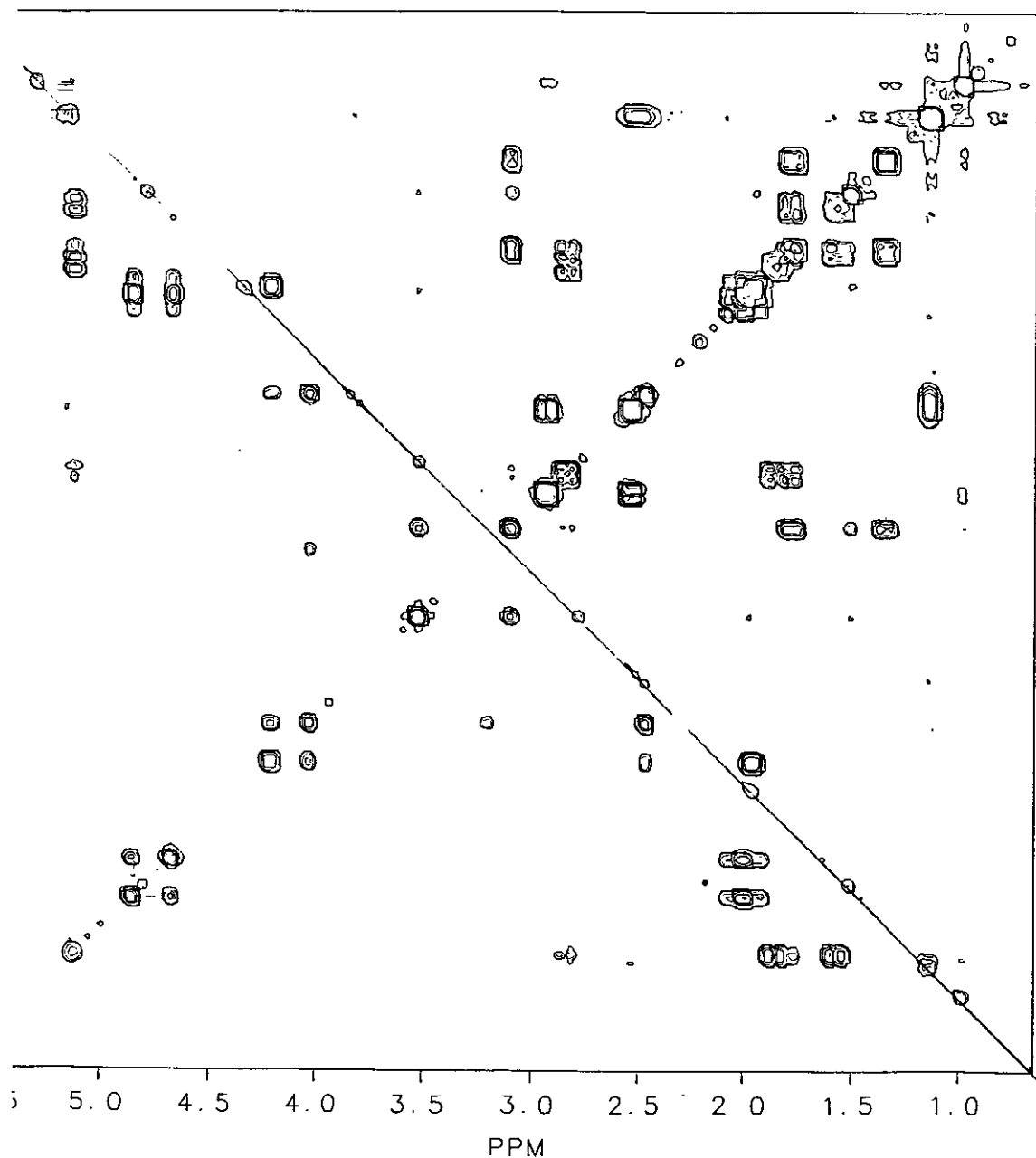
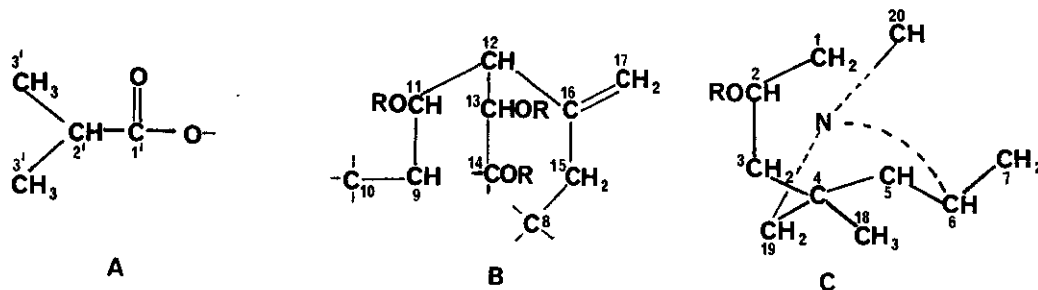


Figure 3. HOMCOR (COSY) experiment of Guan-fu base Z (6). The ^1H -spectrum appears along the lower-left/upper-right diagonal while the lower-right/upper left diagonal may contain artifacts.

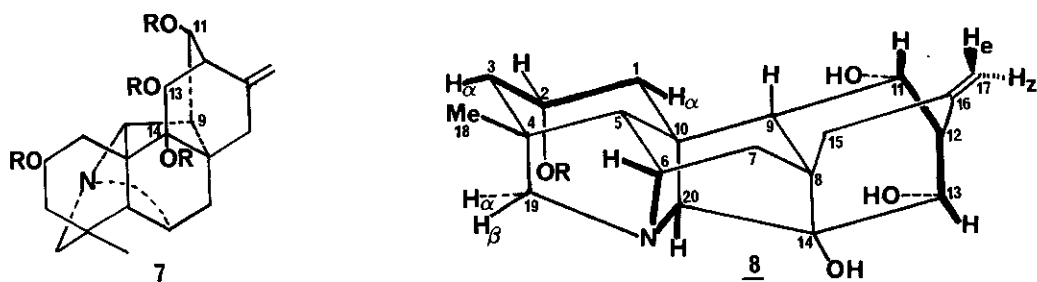
mass spectrum at m/x 328(83)(M-87) further confirms that Guan-fu Base Z contains an isobutyrate group A.

Similarly, the 16,17-alkene is correlated with the allylic 15-methylene and the 12-methine. The latter is associated with the 2°-oxygen functionalities at 11 and 13 and the 3° one at 14, while the former is near the quaternary center at 8. The 3°-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 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° 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 sadosine²³. 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 criteria 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-1 β , H-3 α , and H-7 α) were assigned so as to give the smallest bond separations in the HOMCOR or HETCOR experiments.



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 expected²⁴ 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 alpha⁸ as shown in 6 and 8 since the magnitude of $J_{9,11} = 8.7$ implies an endo-endo relation of H-9 and H-11²⁵. 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 strong, nonallylic, 4J -HOMCOR's are for the planar "W"'s H-6 to H-20 and H-1 α to H-3 α as indicated in 8²⁶. 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 expected²⁷ 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-11²⁷. 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 performed¹¹, H-19 α and H-17 α 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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REFERENCES

1. S.W. Pelletier and N.V. Mody, The Alkaloids, 1979, 17, 1.
2. S.W. Pelletier and N.V. Mody, The Alkaloids, 1981, 18, 99.
3. H.-C. Kao, F.-H. Yo, and J.-H. Chu, Yao Hsueh Hsueh Pao, 1966, 13, 186; Chem. Abstr., 1966, 65, 3922g.
4. (a) J.-H. Liu, H.-C. Wang, Y.-L. Kao, and J.-H. Chu, Chung Ts'ao Yao, 1981, 12, 1; (b) Chem. Abstr., 1981, 95, 138450q; (c) S.-Z. Chen, X.-Q. Ma, and Y.-P. Wang, Acta Chim. Sinica (Translation), 1984, 42, 1.
5. T.E. Monakhova, T.F. Platonova, A.D. Kuzovkov, and A.I. Shreter, Khim. Prirodn. Soedin., Akad. Nauk Uz. SSR, 1965, 113; Chem. Abstr., 1965, 63, 7347f.
6. Y.-L. Chu and J.-H. Chu, Heterocycles, 1982, 17, 607.
7. There is some inconsistency/ambiguity in the representations of the stereochemistry of the oxygen functions at C-11 and C-13 of 2 and 3 in the literature. The structures used in this paper are those given in references 4a and 4b for Guan-fu bases G and A, respectively, and not those in reference 6 which show an incorrect beta C-13 oxygen function (to the right as drawn according to the convention of Pelletier for other hetisine-type alkaloids⁸). Reference 6 also shows the incorrect beta-(solid line) instead of alpha-(dotted line) orientation for the C-11 acetoxy group⁹ of Guan-fu base A. Finally, the methiodide of Guan-fu base

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 5¹⁰.

8. S.W. Pelletier, J.A. Glinski, K.I. Varughese, J. Maddry, and N.V. Mody, Heterocycles, 1983, 20, 413.
9. It is probable¹¹ that Guan-fu base A is actually 4, the 2,13- not the 2,11-diacetate of 14-hydroxyhetisine as claimed^{4a,4b}.
10. K.I. Varughese and S.W. Pelletier, J. Nat. Prod., 1984, 47, 470.
11. M.G. Reinecke, D.E. Minter, D.C. Chen, and W.M. Yan, manuscript in preparation.
12. D.C. Chen and W.M. Yan, unpublished results. Guan-fu Base Z was obtained as white needles, mp 230-231°C from silica gel chromatography of the chloroform extracts of the neutralized (pH 8-9) 1% HCl extracts of the original ethanol extract of the plant.
13. S.W. Pelletier and Z. Djarmati, J. Am. Chem. Soc., 1976, 98, 2626; N.V. Mody and S.W. Pelletier, Tetrahedron, 1978, 34, 2421.
14. A. Bax, "Two-Dimensional Nuclear Magnetic Resonance in Liquids," Delft University Press and D. Reidel Publishing Co., Boston, 1982.
15. R. Benn and H. Gunther, Angew. Chem. Int. Ed. Engl., 1983, 22, 350.
16. J.N. Shoolery, J. Nat. Prod., 1984, 47, 226.
17. L.F. Johnson and W.C. Jankowski, "Carbon-13 NMR Spectra, A Collection of Assigned, Coded, and Indexed Spectra", Wiley-Interscience, Inc., New York, N.Y., 1972.
18. L.M. Jackman and S. Sternhell "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd Ed. Pergamon Press Ltd. London, 1969.
19. Ref. 18, p. 164, 239
20. J.C. Beloeil, M.A. Delsuc, J.Y. Lallemand, G. Dauphin, and G. Jeminet, J. Org. Chem., 1984, 49, 1797.
21. W.F. Reynolds, R.G. Enriquez, M.A. Chavez, A. L. Silba, and M.A. Martinez, Can. J. Chem., 1985, 63, 849.
22. N.H. Fischer, E.J. Oliver, and H.D. Fischer, Prog. Chem. Org. Nat. Prod., 1979, 38, 47.

23. T. Okamoto, H. Sanjoh, K. Yamaguchi, Y. Iitaka, and S. Sakai, Chem. Pharm. Bull. (Tokyo), 1983, 31, 360.
24. Ref. 18, p. 288
25. Ref. 18, p. 280ff
26. Several other nonallylic 4J and 5J correlations are also observed, but they are relatively weak. Two of the latter ($H^{1A}-H^6$, H^2-H^{18} , and $H^2-H^{19\beta}$) show planar or extended W conformations as described by A.P. Marchand in "Stereochemical Applications of NMR Studies in Rigid Bicyclic Systems", Verlag Chemie, Deerfield Beach, Fla., 1982, p. 193ff.
27. Ref. 18, p. 238ff

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