Tetrahedron Vol 42, No 24, pp 6621 to 6626, 1986
Printed in Great Britain

0040-4020/86 $5.00 + 00

Alkaloids of the Chinese Herb Guan-Bai-Fu (*Aconitum koreanum*); Guan-Fu Bases Y and A

Manfred G. Reinecke* and David E. Minter
Department of Chemistry, Texas Christian University
Fort Worth, Texas 76129, U.S.A.

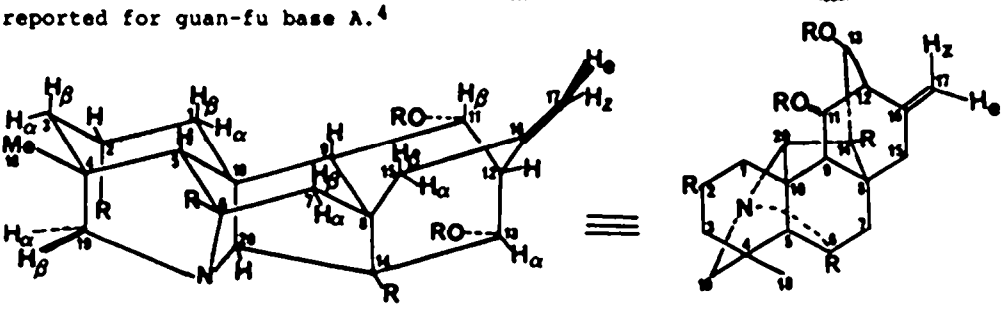
De Chang Chen and Wen Mei Yan
Beijing College of Traditional Chinese Medicine
Beijing, Peoples Republic of China

(*Received in USA* 15 *August* 1986)

Abstract

A new alkaloid, guan-fu base Y, has been isolated from *Aconitum koreanum*(Levl.)Raipaics and shown to be 2-acetoxy-14-hydroxyhetisine (2) from its nmr (^{1}H, ^{13}C) and mass spectra. A complete assignment of nmr peaks was made with the help of NOE and 2-D experiments which also suggest a revised structure (3) for guan-fu base A (4).

Structures of diterpenoid alkaloids continue to be unraveled at a rapid pace[1] based largely on the utility of ^{13}C-nmr spectroscopic correlations with model compounds.[2] Recently we reported the use of 2-D nmr methods to determine the structure of guan-fu base Z (1), an alkaloid of the hetisine type, isolated from the Chinese herbal drug guan-bai-fu [*Aconitum koreanum*(Levl.)Raipaics].[3] This paper describes the structure and spectral assignments of a new alkaloid from this herb, guan-fu base Y (2), and also suggests a revision (3) for the structure (4) previously reported for guan-fu base A.[4]



Compound	R^2	R^6	R^{11}	R^{13}	R^{14}
1	OCO-isoC_3H_7	H	H	H	OH
2	OAc	H	H	H	OH
3	OAc	H	H	Ac	OH
4	OAc	H	Ac	H	OH
5	OAc	H	Ac	Ac	OH
6	OAc	H	Ac	Ac	OAc
7	=O	OH	H	Ac	H

Guan-fu base Y (2) was obtained as colorless crystals, mp 218-219° (acetone) from the gradient elution (cyclohexane-ethyl acetate-diethylamine), silica gel chromatography of the chloroform extracts of the basified (pH 8-9) 1% HCl extracts of the original ethanol extracts of ground *A. koreanum*. The ^{13}C-spectrum (Table I) of 2 is very similar to that of guan-fu base Z (1) except that the isobutyryl carbon resonances of the latter are replaced by those of an acetyl group. This interpretation

is supported by the mass spectrum of 2 which has ions at m/e 387(38) (M^+ for $C_{22}H_{29}NO_5$) and 328(70) (M-OAc) and also shows a striking similarity to that of guan-fu base Z (1) in its fragmentation pattern (Table II). Finally, the skeletal ^1H-spectrum of 2 (Table III) differs only slightly from that of 1 as might be expected for the replacement of an isobutyryl by an acetyl group. Carbon-hydrogen connectivities were verified by means of a ^1J HETCOR experiment[5] whose results were identical to those obtained for 1[3] except for those due to the change in ester groups. All these data convincingly demonstrate that guan-fu base Y is 2-acetoxy—14-hydroxyhetisine (2).

Spectral Parameters of Guan-fu Bases Z(1) and Y(3)

Table I. ^{13}C-nmr [a]

Carbon	1[b,c]	2
1	31.37t	31.14t
2	69.56d	70.00d[c]
3	36.71t	36.56t
4	37.61s	37.62s
5	59.93d	59.95d[c]
6	63.03d	63.06d[c]
7	31.96t	31.96t
8	44.30s	44.28s
9	53.51d	53.52d[c]
10	46.33s	46.37s
11	76.04d	76.01d[c]
12	52.66d	52.49d[c]
13	79.95d	79.88d[c]
14	80.23s	80.26s
15	31.06t	31.04t
16	144.65s	144.56s
17	108.17t	108.24t
18	29.70q	29.70q
19	62.98t	63.03t
20	69.12d	69.16d[c]
1'	176.51s	170.59s
2'	34.41d	21.75q
3'	19.08q	

[a] in $CDCl_3$, ppm relative to TMS = 0; [b] ref. 3; [c] multiplicities verified by a DEPT experiment (ref. 5).

Table II. Mass Spectra [a]

ion	1	2
M^+	415 (38)	387 (38)
M-17	398 (68)	370 (80)
M-28	387 (66)	359 (81)
M-45	370 (100)	342 (100)
M-56	359 (55)	331 (54)
328	(83)	(70)
312	(68)	(78)
310	(36)	(36)
300	(23)	(26)
272	(27)	(24)
146	(56)	(54)
105	(53)	(61)
94	(40)	(41)
91	(59)	(67)
79	(43)	(39)
71	(44) (C_3H_7CO)	(9)
55	(50)	(42)

[a] Taken at 70eV on a Finnegan 1020 OWA instrument; selected ions are reported as m/e (rel.int.) and include all those above m/e 45 with rel.int. $\geq$ 50.

The availability of spectral data for a second alkaloid of this type permits a partial rationale for the mass spectral fragmentation patterns of 1 and 2 as given in Figure 1. Each path begins with α-cleavage of the nitrogen-centered radical cation A and follows reasonable fragmentation mechanisms.[6] Particularly noteworthy is the M-45 base peak which does not appear to have been reported for other hetisine-type alkaloids and may be indicative of the unique 14-hydroxyl function as shown in Figure 1. Confirmation of this hypothesis as well as the other fragmentation paths awaits exact mass, isotope, and metastable ion studies.

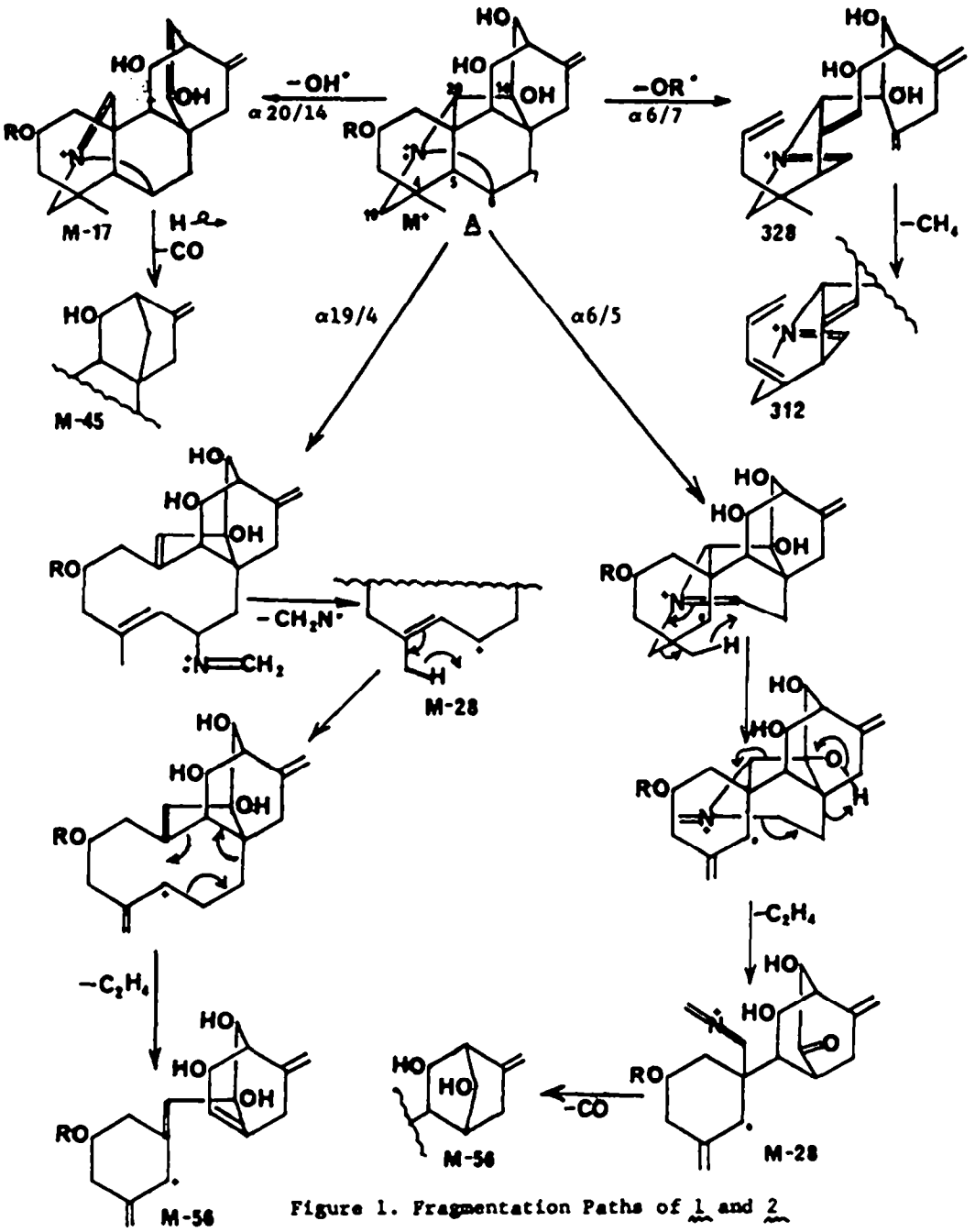


Figure 1. Fragmentation Paths of 1 and 2

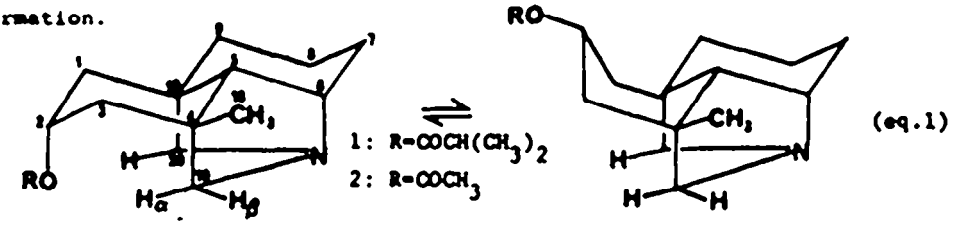
The ^{1}H-spectrum of guan-fu base Y (2) displays significantly better resolution than that of 1 revealing several additional structurally significant couplings, (Table III). For example, the equatorial (α) nature of the low-field proton on C-1 is demonstrated by its three different couplings ($^2J_{1\alpha,1\beta}$, $^3J_{1\alpha,2\beta}$, $^4J_{1\alpha,3\alpha}$) just as the axial (β) nature of the high-field proton on C-3 is supported by the observation of only two couplings ($^2J_{3\alpha,3\beta}$ and $^3J_{3\alpha,2\beta}$). Similarily, the sharp, evenly-spaced (1.9-2.4Hz), eight-line multiplet ($W_{1/2}$ = 9.5Hz) for H-2 is consistent with the four similar coupling constants (to 1α, 1β, 3$\alpha$and 3β) expected for an equatorial (β) rather than for an axial conformation. Finally, the relative magnitude of $^3J_{1\alpha,2\beta}$ (~2Hz) and $^3J_{1\beta,2\beta}$ (4.6 Hz) is in agreement with the former being an equatorial/equatorial and the latter an axial/equatorial interaction.[7]

Table III. Skeletal ^{1}H-parameters for Selected Hetisine Alkaloids[a]

Proton	1[b,c]	2[c]	3[f]	5[h]	6[h]
1α	2.85d(15.7)	2.91ddd(15.9,2,2)			
1β	1.86m	1.86dd(15.8,4.6)			
2	5.13m	5.14dddd($W_{1/2}$=9.5)	5.06m	5.04m	4.96br.s
3α	1.77m	1.86m[d]			
3β	1.59dd(15.4,4.1)	1.59dd(15.5,4.9)			
5	1.52s	1.55s			
6	3.11br.s	3.13br.m			
7α	1.80m	1.82dd(13.9,3.3)			
7β	1.37dd(13.9,2.2)	1.39dd(13.9,2.4)			
9	1.98m	1.99d(8.9)			
11	4.22d(8.7)	4.23br.d(9)	4.20d(8)	5.04m	4.96br.s.
12	2.47m	2.51br.d(3)	2.00[g]		
13	4.04br.s	4.07dd(2.4,2.4)	4.60br.s	4.80br.s	4.72br.s
15α	2.0m	2.08ddd(?,2.0,2.0)[e]			
15β	2.0m	1.99ddd(17.9,2.5,2.5)			
17z	4.86br.s	4.89dd(1-3)	4.85br.s	4.94br.s	4.81br.s
17e	4.68br.s	4.70dd(1-3)	4.79br.s	4.88br.s	4.77br.s
18	1.01s	1.02s	0.96s	0.96s	0.94s
19α	2.95d(12.2)	2.98d(12)			
19β	2.52d(12.2)	2.57d(12)			
20	3.53s	3.55d(1.2)	3.38s	3.30s	3.96s

[a]At 300MHz in $DCCl_3$ with D_2O exchangable proton omitted unless otherwise noted; splittings read directly from spectrum assuming first order coupling; [b]ref. 3; [c]chemical shifts of overlapping peaks estimated from ^{1}J-HECTOR experiments; [d]probably a ddd but partially obscured by H-1β and H-7α; [e]the lowest field portion of this multiplet is obscured by the acetate methyl at 2.07 ppm; [f]ref. 4, 100 MHz, $CDCl_3$; H-11 and H-13 reassigned; cf. text; [g]assigned by decoupling experiment in ref. 4; cf. text; [h]ref. 4, 100 MHz, CCl_4; H-2, H-11, and H-13 assigned as in text.

One curious difference involving A-ring couplings was noted in the HOMCOR[5] 2-D spectra of 1 and 2. While some twenty strong correlations are common to both alkaloids, the one from 2β-3β is present only in 1 and the one from 3β-19β only in 2. One possible rationale for this difference might be variations in specific proton relaxation times due to changes in the chair-boat equilibrium (eq. 1) arising from the considerably greater bulk of an axial isobutyryl (1) compared to an acetyl (2) group. An inspection of models reveals considerable interference between the 2α-substituent and the 19α- and 20-proton in the chair but not the boat conformation.

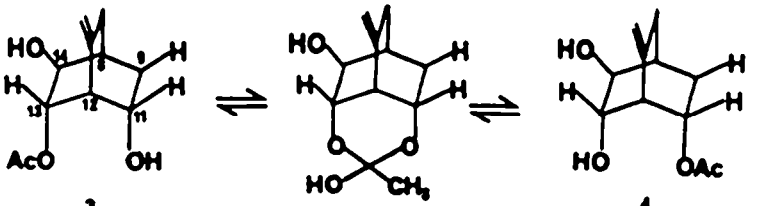


(eq.1)

Another noteworthy feature of the ^{1}H-coupling of guan-fu base Y (2) is the clean doublet of 8.9Hz for H-9 which indicates a dihedral angle with the vicinal H-11β near 0°.[7] A reciprocal coupling is seen in the H-11 doublet (J=9.0) which, however, shows additional couplings in the 1-3Hz range due to $^3J_{11\beta,12}$ and $^4J_{11\beta,13\alpha}$. These observations are consistent with the assigned stereochemistry of the 11α- and 13β-OH groups in both alkaloids 1 and 2 and are similar to those in related hetisine-type alkaloids such as geyeridine (7) and its congeners.[8]

In these latter alkaloids, but not 1 and 2, H-13 as well as H-11 display a coupling of 9-10Hz due to the presence of an eclipsed vicinal proton. Replacement of H-14 with an OH leaves H-13 with only the much smaller (1-3Hz) couplings of $^3J_{13\alpha,12}$ and $^4J_{13\alpha,11\beta}$ resulting in the expected[8] dd for guan-fu base Y (2) or a broad singlet in the less resolved spectrum of guan-fu base Z (1).[3] By the same token the observation of a doublet at 4.20δ with J = 8Hz for the CHOH group of guan-fu base A[4] clearly places this functionality at the 11-, not the 13-position as claimed, and the structure must therefore be revised from 4 to 3. The structures of guan-fu base C (5) and its acetate (6) are correct as postulated,[4,9] since the 9-10Hz coupling for the 11-CHOAc is obscured by overlap with the 2-CHOAc as assigned in Table III.

The basis for the earlier claim[4] that guan-fu base A was the 2,11-(4) rather than the correct 2,13-diacetate (3) of 14-hydroxyhetisine was based on a positive periodic acid test for vicinal diols. In view of the nmr-based reassignment discussed above it must be concluded that this reaction is accompanied by deacetylation or more probably an O-13 to O-11 acetyl migration (eq. 2) which generates the cleavable vicinal diol 4. Similar acetyl migrations under mild conditions are well-known.[10]



3 4 (eq.2)

Finally, it has been possible to assign the nonequivalent methylene protons at C-17 and C-19 as shown in Table III by means of NOE experiments. Irradiation at δ= 1.02 (H-18) significantly enhances peaks at 3.13 (H-6), 1.57 (H-5 and 3β) and 2.57 (H-19β) but not at 2.98 (H-19α). An inspection of Dreiding models clearly shows that H-19β is much closer (2.0Å) to H-18 than H-19α (3.3Å) and must therefore be the 2.57 peak. The enhancement of H-3β but not H-3α is perhaps also noteworthy since these protons are symmetrically disposed around the 18-CH_3 in the chair but not the boat form of ring A in which H-3β is much closer (2.0 vs. 3.3Å) thereby once again suggesting the possibility of the conformational equilibrium in eq. 1.

The second NOE experiment involved irradiating at δ= 2.50 (H-12) and observing enhancements at 4.07 (H-13), 4.23 (H-11), and 4.89 (H-17z) but not at 4.70 (H-17e). Once again models show the H-12 is much closer to H-17z (2.4Å) than to H-17e (3.7Å) thereby substantiating the assignment of the lower field resonance to the olefinic proton nearest the bridgehead. A similar conclusion has been reached for other 2-alkylidene[2.2.2]bicyclooctane systems[11] and for the related alkaloid geyeridine (7) on which the reverse NOE experiment was performed, i.e. irradiation of 17z and

enhancement of H-12.[8] A possible contradiction to these general assignments might be claimed for guan-fu base A (3) which upon irradiation at δ = 2.0(H-12) causes decoupling at 4.20 (H-11), 4.60 (H-13), and 4.85, but not at 4.79.[4] If it is assumed that $J_{transoid} > J_{cisoid}$, as is usually the case in such allylic systems,[12] then H-17e, not H-17z, would be the decoupled, lower-field olefinic proton. This apparent contradiction can be resolved if, as in 1 and 2 (Table III), H-15 of 3 also absorbs at δ = 2.0, and it is this irradiation, not that of H-12, which is responsible for the preferential decoupling of the transoid low-field olefinic proton, i.e., H-17z. Furthermore, Dreiding models show that the dihedral angle between H-12 and either H-17 is near 0°, and hence coupling is neither expected[12] nor is it observed in the HOMCOR spectra of 1[3] or 2. On the other hand coupling between either H-17 and either H-15 is predicted by their 60° dihedral angle, observed in both of the above HOMCOR spectra, and supported by the apparent ddd multiplicity of the H-15 proton peaks.

The methylene protons at positions 7 and 15 were assigned with the lower field member of each pair in the α-configuration due to the expected deshielding by the 1,3-diaxial-like 14-OH group.[13] Since the ^{1}H-nmr of H-15 is possibly not first order, and is partially obscured by the acetate methyl group, some ambiquity must remain in this assignment, however.

ACKNOWLEDGEMENT

This research was supported by the TCU Research Fund and the Tarrant County Charitable Foundation Trust. The Varian XL-300 nmr was purchased with a generous gift from Dr. Malcolm K. Brachman. Translations of the Chinese articles and of correspondence between our laboratories was kindly provided by Mr. Cheng-yi Qian.

REFERENCES

1. X.T. Liang, Pure & Appl. Chem., 58, 711 (1986).
2. S.W. Pelletier and Z. Djarmati, J. Am. Chem. Soc., 98, 2626 (1976); N.V. Mody and S.W. Pelletier, Tetrahedron, 34, 2421 (1978).
3. M.G. Reinecke, W.H. Watson, D.C. Chen, and W.M. Yan, Heterocycles, 24, 49 (1986).
4. J.H. Liu, H.C. Wang, Y.L. Kao and J.H. Chu, Chung Ts'ao Yao, 12, 1 (1981); Chem. Abstr., 95, 138450q (1981).
5. R. Benn and H. Gunther, Angew. Chem. Int. Ed. Engl., 22, 350 (1983); J.N. Shoolery, J. Nat. Prod., 47, 226 (1984).
6. F.W. McLafferty, "Interpretation of Mass Spectra, 3rd Edition, University Science Books, Mill Valley, Calif., 1980, Chap. 4.
7. L.M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd Edition, Pergamon Press, London, 1969, Chap. 4-2.
8. J.A. Grina, D.R. Schroeder, E.T. Wydallis, F.R. Stermitz, J. Melman, and J.L. Capinera, J. Org. Chem., 51, 390 (1986).
9. S.Z. Chen, X.Q. Ma and Y.P. Wang, Acta Chim. Sinica (Translation), 42, 1 (1984).
10. H.M. Flowers in "The Chemistry of the Hydroxyl Group," Part 2, S. Patai, ed., Interscience Pub., N.Y., 1971, Chap. 18.
11. J.C. Nouls, G. Van Binst, and R.H. Martin, Tetrahedron Lett., 4065 (1967).
12. Ref. 7, p 326-325.
13. J.B. Carr and A.C. Huitric, J. Org. Chem., 29, 2506 (1964).