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STUDIES ON SOME NEW *STEMONA* ALKALOIDS

A DIAGNOSTICALLY USEFUL ^{1}H NMR LINE-BROADENING EFFECT

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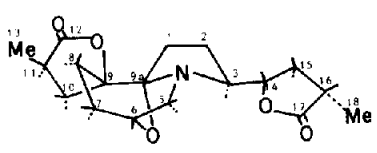
Abstract—Structures of three *Stemona* alkaloids from a Chinese medicinal plant are described. Both stemotinine **1** and isostemotinine **2** isolated from the roots of *Stemona tuberosa* Lour. (Stemonaceae) are shown to be pentacyclic compounds containing two α-methyl-γ-lactone rings; the structure of stemonidine isolated in 1948[1] has also been determined as **3**. Structure elucidations are based on detailed ^{1}H- and ^{13}C NMR studies. A preliminary account is given on a line-broadening effect of ^{1}H NMR signals which are in spatial proximity with incipient N^+–D bonds.

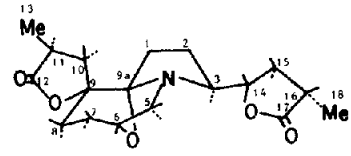
The roots of *Stemona tuberosa* Lour. and related *Stemona* species (Stemonaceae) are used in Chinese medicine as insecticides and/or anticough agents. So far the structures of eleven *stemona* alkaloids, i.e. stemonine, protostemonine, tuberostemonine, tuberostemonine A, oxotuberostemonine, stenine, stemonine, stemofoline, stemonamine, isostemonamine, and croomine, have been determined, mostly by X-ray analyses.[2–7] In this paper we wish to report the structures of stemotinine **1** and isostemotinine **2** which were isolated from the roots of *Stemona tuberosa* Lour., collected in Yunnan Province in China, and that of stemonidine **3**, isolated from another *Stemona* species;[1] we also report a diagnostically useful ^{1}H NMR observation which played a crucial role during structural studies.

The molecular formula of stemotinine **1**, $C_{18}H_{25}NO_5$, M^+ at *m/z* 335.1751 (Calc 335.1730) requires seven degrees of unsaturation. The presence of two singlets at 179.2 and 179.7 ppm in the ^{13}C NMR CO region and a strong IR absorption around 1764 cm^{-1} suggested that two γ-lactones were present. This evidence together with the lack of olefinic carbons (NMR), lack of NH and OH groups (IR), and lack of UV-absorbing chromophores (end absorption in UV) indicated that stemotinine consisted of a pentacyclic skeleton incorporating two γ-lactones, one ether and one *t*-amine.

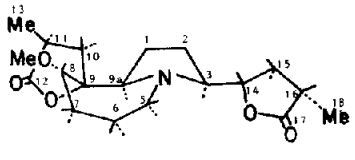
Detailed ^{1}H NMR (360 MHz) studies utilizing differential NOE, spin tickling, two dimensional J techniques,[8] etc, led to full clarification of the proton signals (Table 1); these data in conjunction with ^{13}C NMR results led to the four partial structures **5–8** (Fig. 1). Of the four quaternary carbons present in stemotinine, two are due to the lactones and the remaining two are assignable to moieties **7** and **8**. Upon addition of DCl (far less mole equiv) to the $CDCl_3$ solution of stemotinine, the NMR signals of protons close to N in partial structure **5** (see also **1**) underwent the following downfield shifts (and line-broadening—see below): 3α-*H* + 0.09, 5α-H + 0.12; 5β-H + 0.18; 6-H + 0.07; 14-H + 0.32. In structure **5**, 14-C and 17-O are linked to form the γ-lactone which is apparent in the M^+–$C_5H_7O_2$ peak; this MS fragment is



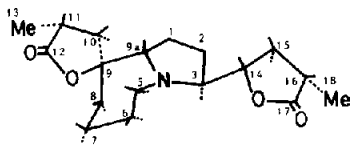
1 Stemotinine



2 Isostemotinine



3 Stemonidine



4 Croomine

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Table 1. ^{1}H NMR data (360 MHz) of stemotinine, isostemotinine, stemonidine and croomine in $CDCl_3$

No.	Stemotinine 1	Isostimotinine 2
1α,β	1.86 & 1.91 m	1.88 & 1.92 m
2α,β	1.72 & 1.98 m	1.60 & 2.15 m
3α	2.86 ddd 10.8(2β),8.8(14β),5.8(2α)	2.93 ddd 10.8(2β),7.8(14β),6.1(2α)
5α	3.00 ddd 10.7(5β),6.3(6),1.4(7α)	3.04 dd 10.4(5β),6.3(6)
5β	3.22 d 10.7(5α)	3.20 d 10.4(5α)
6	4.59 m 6.3(5α),2.0(7α),2.0(7β),1.4(8α)	4.68 ddd 6.3(5α),2.0(7α),2.0(7β)
7α	1.81 m 13.5(8β),12.6(7β),5.9(8α)	
7β	1.62 bdd 12.6(7α),5.4(8β),1.8(8α)	
8α	1.55 ddt 13.5(8β),5.9(7α),1.8(7β),1.8(6)	
8β	2.34 dt 13.5(8α),13.5(7α),5.4(7β)	
10α	2.61 dd 14.6(10β),11.6(11)	2.10 dd 13.1(10β),10.0(11)
10β	1.70 dd 14.6(10α),6.3(11)	1.71 dd 13.1(10α),12.6(11)
11	2.81 ddq 11.6(10α),7.7(11-Me),6.3(10β)	2.80 ddq 12.6(10β),10.0(10α),7.7(11-Me)
11-Me	1.34 d 7.7(11)	1.28 d 7.7(11)
14β	4.26 ddd 11.3(15α),8.8(3α),5.4(15β)	4.14 ddd 11.3(15α),7.8(3α),5.4(15β)
15α	1.48 ddd 12.6(16β),12.6(15β),11.3(14β)	1.58 ddd*
15β	2.36 ddd 12.6(15α),9.0(16β),5.4(14β)	2.36 ddd*
16β	2.67 ddq 12.6(15α),9.0(15β),7.5(16-Me)	2.66 ddq*
16-Me	1.26 d 7.5(16β)	1.28 d*

No.	Stemonidine 3	Croomine 4
3α	3.30 ddd 7.4(2β),6.8(2α),6.8(14β)	3.36 ddd 7.4(2β),6.8(2α),6.8(14β)
5α,β	3.10 m	3.12 m
8β	3.22 dd 6.8(7α),2.4(7β)	
9a	3.77 dd 8.0(1α),6.6(1β)	3.50 dd 8.2(1α),7.8(1β)
10α	2.47 dd 14.0(10β),11.0(11)	2.45 dd 13.5(10β),10.9(11)
10β	1.63 dd 14,0(10α),8.0(11)	1.65 dd 13.5(10α),7.9(11)
11	2.71 ddq 11.0(10α),8.0(10β),7.8(11-Me)	2.72 ddq 10.9(10α),7.9(10β),7.8(11-Me)
11-Me	1.31 d 7.8(11)	1.32 d 7.8(11)
14β	4.38 ddd 11.3(15α),6.8(3α),5.4(15β)	4.32 ddd 11.3(15α),6.8(3α),5.4(15β)
15α	1.50 ddd*	1.51 ddd*
15β	2.38 ddd*	2.37 ddd*
16β	2.62 ddq*	2.61 ddq*
16-Me	1.27 d*	1.27 d*
8-OMe	3.40 s	

* J value of 15α-,15β-,16β-H and 16-Me of 2,3 and 4 are the same as those of 1.

characteristic of *Stemona* alkaloids, all of which have an α-methyl-γ-lactone linked α to a pyrrolidine ring.[9]

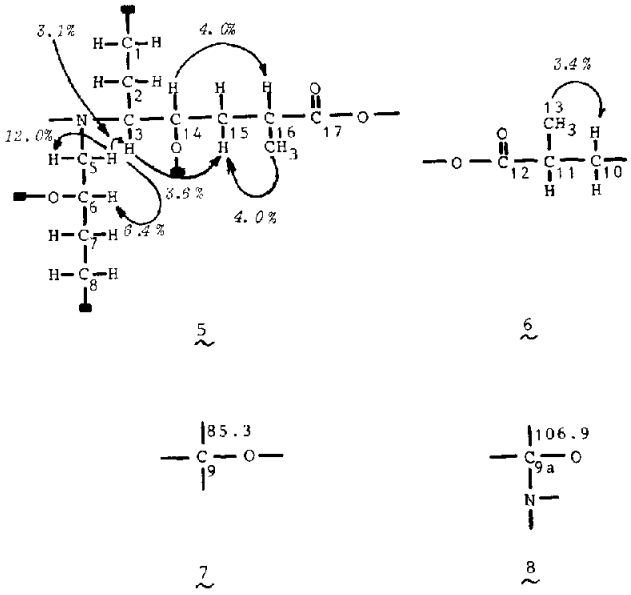
A 3.6% NOE was observed at 15α-H upon irradiation of 3α-H, i.e. the two protons are in proximity, and this shows that the most abundant population of the pendant γ-lactone is as depicted in **1**. It was noted that upon addition of a *trace* of DCl to the NMR $CDCl_3$ solution, the signals of protons *spatially close to the nitrogen lone pair*, i.e. in this case 14-H, underwent dramatic line broadening, whereas the shapes of other nearby protons, 3α-, 5α-, 5β-, and 6-H, were affected much less (see below).

The other γ-lactone comprises a spiro lactone system which is supported by another prominent MS fragment peak (M^+–$C_5H_7O_2$–$C_5H_6O_2$). The fact that one of the 10-H's (10α-H) is at the low-field of 2.61 ppm, which contrasts the 1.70 ppm shift of its geminal 10β partner, is attributed to the anisotropic effect of the oxygen, and determines the C-9 configuration. The 10β-H undergoes a 3.4% NOE enhancement when 13-H (11-Me) is irradiated; the 11-Me hence is β-oriented. The conformation of the perhydroazaazulene ring is as depicted on the basis of the observation of two W-type long-rang couplings between 5α-H/7α-H (1.4 Hz) and between 6-H/8α-H (1.4 Hz). The 5α-H is involved in NOE with all three neighbouring protons, 5β-H, 3α-H and 6-H; it is the 3.1% NOE between 5α-H/3α-H which initially enabled one to interrelate the two proton systems flanking the t-N in structure **5**.

The MS, IR, CD and ^{13}C NMR data of isostemotinine

NOE's (%) (in $CDCl_3$, $C_6D_6^a$)

Stemotinine 1			Isostemotinine 2			Stemonidine 3		
irr.H	observed		irr.H	observed		irr.H	observed	
3α	15α	3.6	3α	15α	2.7	3α	15α	4.0[a]
5α	3α	3.1	5α	3α	3.5	5α	9a	2.9[a]
5α	5β	12.0	5α	5β	8.2	OMe	8β	7.0[a]
5α	6	6.4	5α	6	6.8	10β	8β	5.2[a]
11-Me	10β	3.4	11-Me	10β	3.4	11	10α	2.3[a]
14β	16β	4.0	11	10α	2.7	14β	16β	4.7[a]
16-Me	15α	4.0	14β	16β	3.4	16-Me	15α	2.2
			16-Me	15α	3.1			



5 6 7 8

Fig. 1. (NOE's; Partial structures of 5–8).

2, $C_{18}H_{25}NO_5$, M^+ at *m/z* 335.1742 (Calc 335.1730), closely resembled those of stemotinine **1**. The ^1H NMR signals are also similar except for those due to 10-H's: in **1** they are at 2.61(dd)/1.70(dd) whereas in **2** they are at 2.10(dd)/1.71(dd), namely, the difference in δ values are much smaller in **2** (Table 1; the 7- and 8-H's could not be fully analysed). The closer chemical shifts of the two 10-H's are attributable to lack of the ethereal oxygen anisotropic effect and thus lead to the 9-iso structure **2**. Moreover, in **2** the perhydroazaazulene ring has an "8-CH_2-down conformation" in view of the lack of W-type coupling between 5α-H/7α-H and 6-H/8α-H. This conformation is further supported by the *dramatic broadening of the* 10α-*H signal*, as well as the 14-H signal, upon addition of *trace* amounts of DCl, while the *signal pattern of* 10β-*H remains practically unaffected.* As described below, this is accounted for by proximity of the 10α-H to the N lone pair electrons; if the perhydroazulene ring adopted the "8-CH_2—up conformation" (as in **1**) the 10α- and 10β-protons would both be similarly situated with respect to the lone pair (see below), which is not the case. The 11-Me configuration rests on the fact that NOE's were observed between 10α-H/11-H and between 10β-H/11-Me.

The structure of stemonidine, m.p. 119°, $[\alpha]_D^{24} - 5.4°$ (acetone), $C_{19}H_{29}NO_5$, M^+ at *m/z* 351.2020 (Calc 351.2044), which was isolated in 1948 as its hydrobromide from *Stemona* sp.[1], has also been determined as **3**. The NMR and other spectroscopic data were similar to those of croomine **4**, the structure of which was recently established by X-ray crystallography.[7] The conspicuous differences between the two were the presence in **3** of the 8-OMe function, δ 3.40, 3H, singlet, and δ 3.22, 1H dd, J = 6.8 and 2.4 Hz, and appearance of a dd signal at δ 3.77 (9a-H, Table 1) in the 1H NMR of stemonidine **3**. The 8-H (3.22 ppm) undergoes a 4.6% NOE enhancement when 10β-H (1.63 ppm) is irradiated; the 8-H hence is β-oriented. Moreover, since a 2.9% NOE was observed at the 9a-H 3.77 ppm peak upon irradiation of the 5α-H 3.05 ppm signal (in C_6D_6), the configuration at C-9a is opposite to that of croomine.

Structure **1**–**3** received additional support from the following observation encountered when DCl was added to the $CDCl_3$ NMR solution. Thus it was noticed that addition of a *trace quantity of* DCl resulted in *extensive broadening of the signals due to protons spatially close to the nitrogen lone pair*; the amount of DCl should be such that the customary down-field shift accompanying formation of ammonium ions is as yet not observable.[10] Thus, in the case of croomine **4** with established structure, line-broadening was observed for 9aβ- and 14-H signals, but the 3α-, 5α- and 5β-H peaks remained essentially unchanged. In stemonidine **3**, extensive broadening was observed with 14-H and 10α-H but not

with 10β-H. Similarly, a trace of DCl induced broadening of the 14-H peak in stemotinine **1**, and the 14-H and 10α-H peaks in isostemotinine **2** (see above), but left all other proton peaks unaffected. We believe that these observations will be of practical value in clarifying subtle structural differences such as those encountered between **1** and **2**, and between **3** and **4**. Further studies are in progress to elucidate the nature of the line-broadeining and to delineate the applicability of the technique as a structural tool.

EXPERIMENTAL

M.ps were determined with a Kofler m.p. apparatus and were uncorrected. UV spectra were recorded on a Shimadzu double beam spectrophotometer UV 210A. IR spectra were run on a Hitachi EPI-G2 spectrometer (in Nujol). Optical rotation were recorded on a Perkin-Elmer Model 141 Polarimeter. CD curves were taken on a JASCO J-20C instrument. High resolution mass spectra were obtained at an ionizing voltage of 70 eV on a JEOL-01SG mass-spectrometer. ^{13}C NMR and ^{1}H NMR spectra were recorded using either JEOL JNM FX-100 (100 MHz) or a Nicolet NT-360 NMR (360 MHz) spectrometer. Chemical shifts are expressed in ppm relative to TMS, and coupling constants (J values) in Hz. The 360-MHz data for 2DJ spectra were obtained with a 90°-t-180°-t-acquisition sequence. Data processing was carried out with standard Nicolet software. The f_2 (chemical shift axis) spectral width was ±2000 Hz over 8K data points; 128 spectra with t incremented by 10.0 ms each time gave an f_1 (J spectra) width of 50.0 Hz with a digital resolution of 0.39 Hz. The steady state NOE difference spectra were obtained at 360 MHz by a method of alternative acquisition technique in order to maximize spectral quality.

Isolation and separation. Air dried fresh roots of *Stemona tuberosa* Lour. collected in Wenshan District, Yunnan Province in China in October 1980, were chopped and extracted with hot EtOH. After concentration of EtOH, the residue was extracted with dil HCl, the latter was washed with $CHCl_3$ and then extracted again with $CHCl_3$ under basic conditions (NH_4OHaq). Removal of the solvent under reduced pressure afforded crude alkaloids. They were dissolved in dil HCl and washed with $CHCl_3$, then extracted with ether and $CHCl_3$ successively under basic conditions. Each extract was then passed through a column of silica gel, eluted with ether under successive addition of acetone (1–8%). Stemotinine (yield *ca* 0.01%) was obtained from the ether extract and isostemotinine (yield *ca* 0.008%) from the $CHCl_3$ extract.

Stemotinine **1** was crystallized from acetone-ether as white prisms, m.p. 207–208°; $[\alpha]_D^{22}$ +91.7° (*c* = 1.1, MeOH); IR 1764, 1190 cm^{-1}; ^{1}H NMR: see Table 1. ^{13}C NMR ($CDCl_3$) δ 179.7(s), 179.2(s), 106.9(s), 85.3(s), 83.1(d), 77.9(d), 71.4(d), 58.0(t), 36.4(t), 35.3(d), 35.1(d), 33.7(t), 31.6(t), 29.8(t), 28.5(t), 28.0(t), 18.5(q), 14.8(q); CD (MeOH) $\Delta\epsilon_{220}$ −0.85; MS *m/z* 335.1751 (11%, M^+, Calc for $C_{18}H_{25}NO_5$ 335.1730), 236.1317 (34%, Calc for $C_{13}H_{18}NO_3$ 236.1286), 138.0888 (10%, Calc for $C_8H_{12}NO$ 138.0918), 124.0872 (100%, Calc. for $C_8H_{12}O$, 124.0888). (Calc for $C_{18}H_{25}NO_5$: C, 64.44; H, 7.52; N, 4.18%. Found: C, 64.29; H, 7.73; N, 3.89%).

Isostemotinine **2** was recrystallized from acetone–ether to give white prisms, m.p. 245–246°; $[\alpha]_D^{22}$ +47.5° (*c* = 0.6, MeOH), IR 1750, 1190 cm^{-1}; ^{1}H NMR: see Table 1. ^{13}C NMR ($CDCl_3$) δ 179.0(s), 178.6(s), 106.6(s), 83.3(s), 82.9(d), 77.3(d), 71.7(d), 58.0(t), 38.2(t), 35.2(d), 34.2(d), 33.9(t), 29.9(t), 29.7(t), 29.4(t), 26.6(t), 15.5(q), 14.9(q); CD (MeOH) $\Delta\epsilon_{210}$ −0.68; *m/z* 335.1742 (M^+), all peaks were superimposed to those of stemotinine.

Stemonidine **3**. Stemonidine hydrobromide,[1] m.p. 278°, was dissolved in dil HCl and extracted with ether under basic conditions to afford the free base, which was recrystallized from ether as white prisms; m.p. 119°; $[\alpha]_D^{24}$ −5.4° (*c* = 0.9, acetone); IR 2780, 1768, 1218 cm^{-1}; ^{1}H NMR: see Table 1. ^{13}C NMR ($CDCl_3$)[11] δ 179.4 (s), 179.3(s), 90.5(s), 85.2(d), 80.1(d), 67.6(d), 63.2(d), 57.9(q), 48.8(t), 35.6(d), 35.0(d), 34.9(t), 34.6(t), 27.1(t), 26.5(t), 25.7(t), 22.4(t), 17.5(q), 14.8(q); CD (MeOH) $\Delta\epsilon_{220}$ −0.21, $\Delta\epsilon_{240}$ +0.16; MS *m/z* 351.2020 (< 1%, M^+, Calc for $C_{19}H_{29}NO_5$ 351.2044), 320.1858 (36%, M–OCH_3, Calc for $C_{18}H_{26}NO_4$, 320.1860), 252.1620 (100%, Calc for $C_{14}H_{22}NO_3$, 252.1598), 154.1240 (6%, Calc for $C_9H_{16}NO$ 154.1232).

Croomine **4**. Croomine picrate[7] was converted to the free base by treatment with 5N HCl and extraction with ether under basic conditions: yellow oil; ^{1}H NMR: see Table 1.

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REFERENCES

[1]J. H. Chu, *Science Record* **2**, 310 (1949); *Chem. Abstr.* **44**, 11030b (1950).

[2]M. Gotz and G. M. Strunz, *Alkaloid*, (Edited by K. Wiesner) MTP, International Review of Sciences, *Organic Chemistry*, Series one, Vol. 9, p. 134–160. Butterworths, London (1975).

[3]O. E. Edwards, *The Alkaloids* (Edited by R. H. F. Manske), Vol. IX, pp. 545–551. Academic Press, New York (1967).

[4]K. Nakanishi, T. Goto, S. Ito, S. Natori and S. Nozoe, *Natural Products Chemistry*, Vol. 2, pp. 292–293. Academic Press, New York (1975).

[5]C. Kuo and T. T. Chu, *Acta Chimica Sinica* **36**, 291 (1978).

[6]H. Iizuka, H. Irie, N. Masaki, K. Osaki and S. Uyeo, *Chem. Comm.* **4**, 125 (1973).

[7]T. Noro, S. Fukushima, A. Ueno, Y. Iitaka and Y. Saiki, *Chem. Pharm. Bull.* **27**, 1495 (1979).

[8a]W. P. Aue, J. Karlan and R. R. Ernst, *J. Chem. Phys.* **64**, 4226 (1976); [b]L. D. Hall and J. K. M. Sanders, *J. Am. Chem. Soc.* **102**, 5703 (1980).

[9]H. Irie, H. Harada, K. Ohno, T. Mizutani and S. Uyeo, *Chem. Comm.* 268 (1970).

[10]A dulute soln of DCl in $CDCl_3$ (4% w/w) was added dropwise (1 drop corresponds to 0.1 mole equiv) to the NMR sample soln. The changes in NMR signals were followed after the addition of each drop. More detailed general applicabilities of this methods are underinvestigation.

[11]We are indebted to Dr. Luu Ban, Institute of Chemistry, Strasburg, for measurements of high-resolution MS and ^{13}C NMR of stemonidine.