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A STUDY OF *STEMONA* ALKALOIDS, III. APPLICATION OF 2D-NMR SPECTROSCOPY IN THE STRUCTURE DETERMINATION OF STEMONININE

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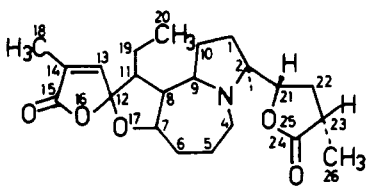
ABSTRACT.—A new alkaloid, stemoninine, obtained from the root of *Stemona sessilifolia*, and its stereochemistry have been elucidated by the analysis of the high resolution ^{1}H-nmr spectrum; ^{1}H,^{1}H-COSY; ^{13}C,^{1}H-COSY shift correlated two-dimensional nmr spectra and ^{1}H long-range shift; and ^{13}C,^{1}H-COLOC- and nOe-correlated 2D-nmr techniques.

Previously we isolated the new alkaloid stemoninine from the root of *Stemona sessilifolia* (Mig.) Frack et Sav. (Stemonaceae; formerly Roxburghiaceae) and elucidated its structure by ir, uv, ^{1}H-nmr, ^{13}C-nmr, and ms data (1,2). In the present paper, we further attempt to elucidate the structure and relative configuration of the molecule using 2D-nmr techniques such as ^{1}H (^{1}H,^{1}H-COSY), ^{13}C-^{1}H (^{13}C,^{1}H-COSY) shift correlations and ^{1}H-^{1}H long-range couplings shift, ^{13}C-^{1}H long-range couplings shift (^{13}C,^{1}H-COLOC) as well as nOe-correlated 2D-nmr techniques (NOESY). From the analysis of these spectral data of stemoninine, the structure **3** is proposed.



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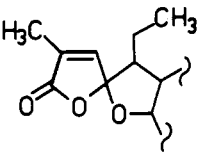

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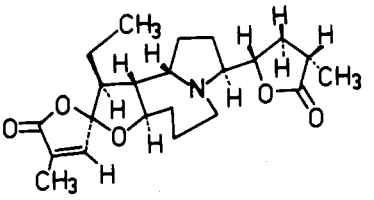

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RESULTS AND DISCUSSION

The molecular formula of stemoninine is $C_{22}H_{31}NO_5$. The molecular ion $[M]^+$ at *m/z* 389.2205 (calcd 389.2202) requires eight degrees of unsaturation. The ^{1}H-nmr signal at 0.77 ppm (t, $J=7.5$ Hz) is due to a methyl group; two methyl groups are at 1.17 ppm (d, $J=7.0$ Hz) and 1.85 ppm (d, $J=2.0$ Hz); one olefinic proton is at 6.59 ppm (d, $J=2.0$ Hz). Two signals at 171.3 ppm and 179.1 ppm in the ^{13}C-nmr carbonyl region and one signal at 113.5 ppm indicate that a ketal group is present, and strong ir absorption around 1765 cm^{-1} suggested that two γ-lactones are present. The analysis of the high resolution ^{1}H-nmr spectrum (400 MHz) ^{1}H,^{1}H-COSY (Figure 1) and ^{13}C,^{1}H-COSY (Figure 2) led to the full clarification of the carbon and proton signals (Table 1). The structure **1** was supported based on 2D long-range ^{13}C,^{1}H-shift correlated spectra (^{13}C,^{1}H-COLOC) (Figure 3), which illustrate important two-, three-, and four-bond couplings in the molecule (Table 2). The carbonyl carbons resonating at 179.1 ppm (C-24) and 171.3 ppm (C-15) correlate with 26-CH_3, 22-H, and 21β-H, and with 18-CH_3 and 13-H. The quaternary (C-12) carbon with $\delta=113.5$

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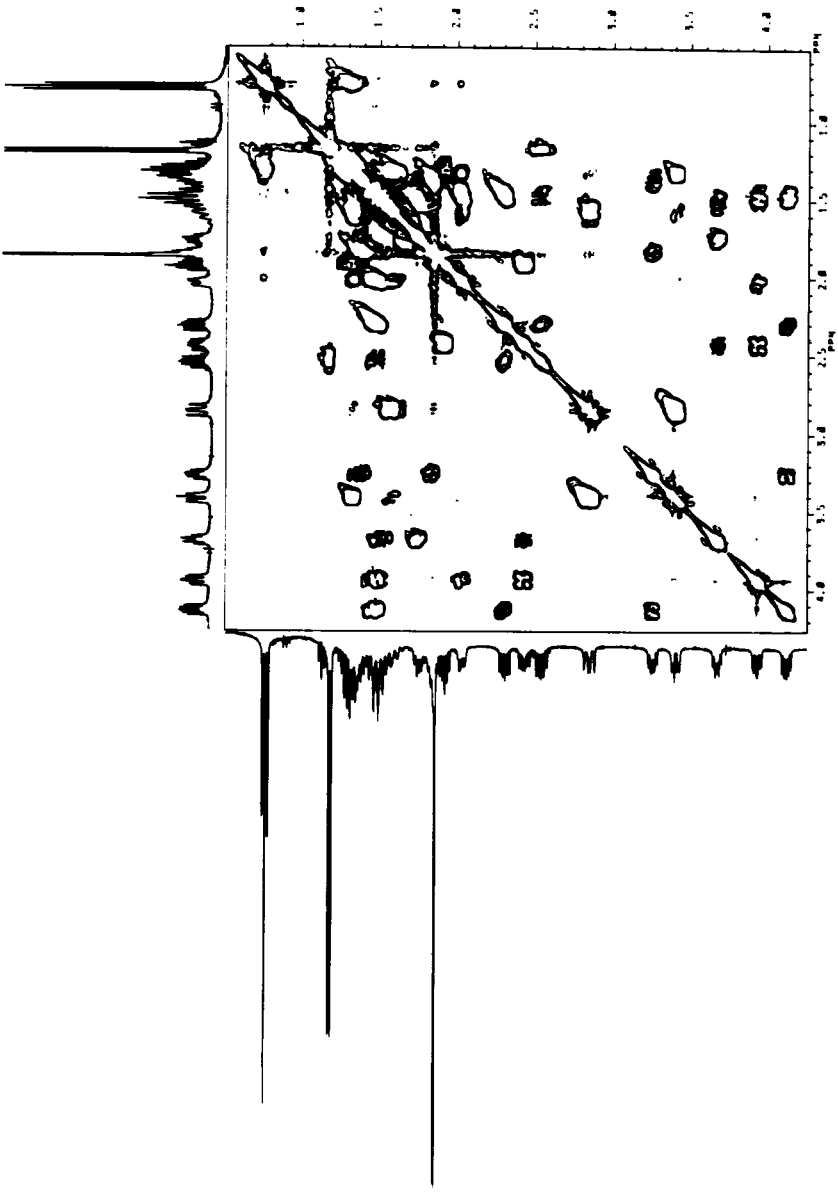

FIGURE 1. ^{1}H shift-correlated 2D-nmr spectrum of stemoninine [3] in $CDCl_3$ (^{1}H, ^{1}H-COSY), 400 MHz.

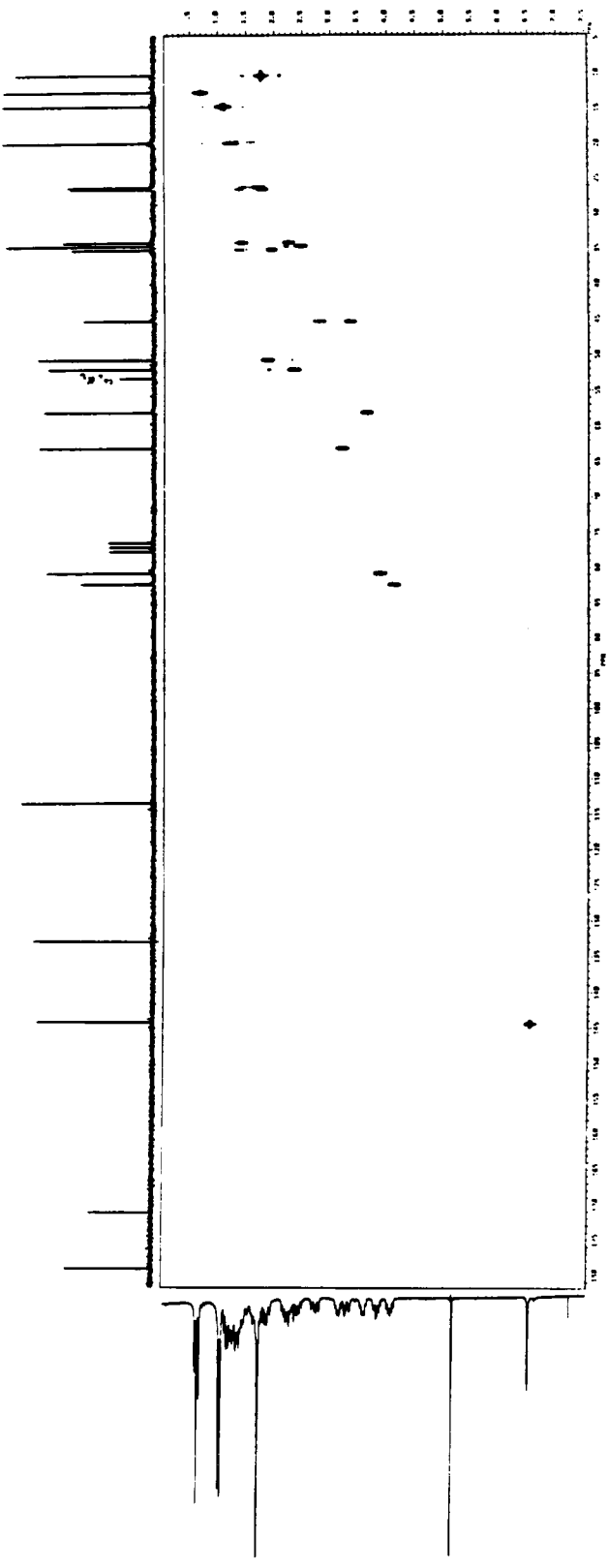

FIGURE 2. ^{1}H, ^{13}C shift-correlated 2D-nmr spectrum of stemoninine [3] in $CDCl_3$ (^{13}C, ^{1}H-COSY), 200 MHz.

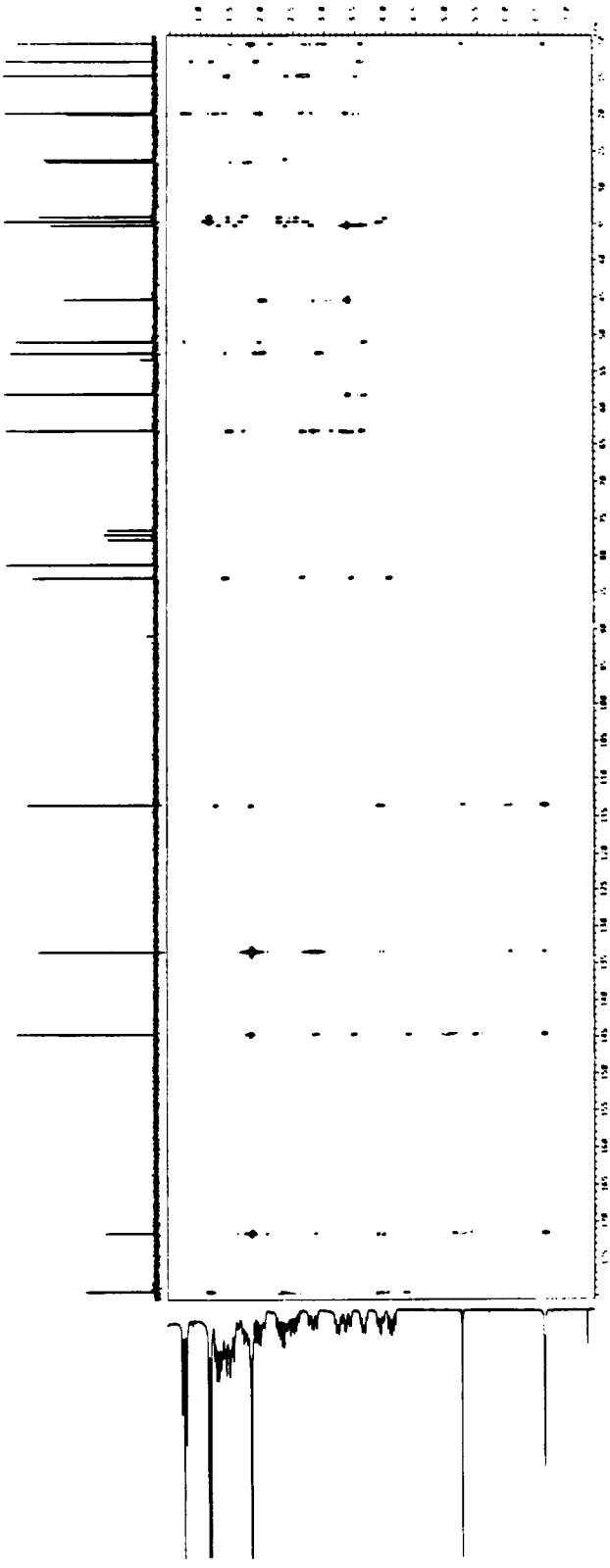

FIGURE 3. 2D long-range ^{1}H, ^{13}C shift-correlated spectrum of stemoninine [3] in $CDCl_3$ (^{13}C, ^{1}H-COLOC), 200 MHz.

TABLE 1. ^{13}C-nmr (100.6 MHz) and ^{1}H-nmr (400 MHz) Data of Stemoninine [**3**].

Carbon	ppm	Proton	ppm	*J* (Hz)
C-1	26.5	1α,β-H	1.35, 1.80	m
C-2	63.4	2α-H	3.25	ddd 5.5 (1β), 7.0 (21β), 10.0 (1α)
C-4	45.6	4α-H	2.86	dd 11.5 (5α), 15.5 (4β)
		4β-H	3.41	dd 9.0 (5β), 15.5 (4α)
C-5	20.2	5α-H	1.57	m
		5β-H	1.33	m
C-6	35.3	6β-H	1.43	m
		6α-H	2.03	brdd 3.5 (7α), 12.0 (6β)
C-7	81.1	7α-H	3.93	ddd 3.5 (6α), 9.5 (6β), 11.0 (8β)
C-8	52.4	8β-H	2.43	ddd 5.5 (11α), 9.5 (9β), 11.0 (7α)
C-9	58.3	9β-H	3.68	dt 6.0 (10α), 6.0 (10β), 9.5 (8β)
C-10	26.3	10α,β-H	1.50, 1.75	m
C-11	51.2	11α-H	1.93	ddd 5.5 (8β), 7.0 (19-H_a), 12.0 (19-H_b)
C-12	113.5			
C-13	144.4	13-H	6.59	d 2.0 (18-CH_3)
C-14	133.5			
C-15	171.3			
C-18	10.3	18-CH_3	1.85	d 2.0 (13-H)
C-19	20.0	19$_a$-H	1.28	m
		19$_b$-H	1.60	m
C-20	12.7	20-CH_3	0.77	t 7.5
C-21	82.4	21β-H	4.14	ddd 5.5 (22α), 7.0 (2α), 10.0 (22β)
C-22	34.1	22β-H	1.43	m
		22α-H	2.31	ddd 5.5 (21β), 9.0 (23β), 12.0 (22β)
C-23	34.7	23β-H	2.54	ddq 7.0 (26-CH_3), 9.0 (22α), 12.0 (22β)
C-24	179.1			
C-26	14.8	26-CH_3	1.17	d 7.0 (23β)

ppm shows long-range couplings with 13-H, 11-H, 19-H, and 7-H, thus indicating the presence of the ketal substructure and an ethyl group linked to C-11. Substructure **2** has also been indicated by metastable ions in the mass spectrum of stemoninine (2).

The assignment of the relative configuration of stemoninine was suggested by the ^{1}H-^{1}H-COSY long-range couplings, by nOe-correlated 2D nmr techniques (NOESY), and by observing the *J*-values in the high resolution ^{1}H-nmr spectrum. Fuyihiko and Akira (3) used the ^{1}H, ^{1}H-COSY long-range 2D nmr for this purpose. The 90-Δ-t_1-45-Δ-t_2 sequence was studied, when Δ (delay time) was set to 350 msec. The cross peaks due to small couplings (less than ca. 1 Hz) are enhanced, while those due to large couplings (more than 3 Hz) are suppressed (Figure 4). The cross peak A is due to the long-range coupling between 11-H, and 20-CH_3. The cross peak B is due to the long-range coupling between 22-H and 26-CH_3, and the cross peak C is due to the long-range coupling between 9-H and 1-H. According to the Karplus equation, we compared our values with the coupling constants of two vicinal protons (8-H, 11-H, $J_{8,11}$ = 5.5 Hz). The corresponding dihedral angle (Φ) between two vicinal protons should be approximately 120°; consequently, the relative configuration of 11-H and 8-H should be *trans*. We observed also the coupling constant between the two vicinal protons at 7-H, 8-H, $J_{7,8}$ = 11.0 Hz and between the two vicinal protons at 8-H, 9-H, $J_{8,9}$ = 9.5 Hz. Their corresponding dihedral angles should be approximately 180° and 0°, respectively. This means that the former should be *trans* oriented and the latter *cis* oriented.

The nOe-correlated spectrum in a contour plot is shown in Figures 5 and 6; the pulse sequence 90-t-90-τ_m-90-t was used. We have used 600 msec and 1000 msec for τ_m; $CDCl_3/CDCl_3$ and C_6D_6/C_6D_6 were used as solvents. The τ_m = 1000 msec seems to

TABLE 2. Cross Peaks of Carbon to Proton in the NOESY of Stemoninine [**3**]; Several Observed Two-; Three-; and Four-Bond ^{13}C-^{1}H Couplings in Stemoninine.

Carbon	ppm	Cross peaks to proton
C-1	26.5	10-H
C-2	63.4	1-H, 2α-H, 4α-H, 4β-H, 9β-H, 22β-H
C-4	45.6	4α-H, 4β-H, 6α-H
C-5	20.2	4α-H, 4β-H, 5β-H, 6α-H, 6β-H, 8β-H
C-6	35.3	4α-H, 4β-H, 5β-H, 6β-H, 7α-H, 8β-H
C-7	81.1	cannot be observed
C-8	52.4	6α-H, 6β-H, 11α-H
C-9	58.3	4β-H, 9β-H
C-10	26.3	1β-H, 8β-H
C-11	51.2	9β-H, 11α-H, 20-CH_3
C-12	113.5	7α-H, 9β-H, 11α-H, 13-H
C-13	144.4	13-H, 18-CH_3
C-14	133.5	7α-H, 13-H, 18-CH_3
C-15	171.3	13-H, 18-CH_3
C-18	10.3	13-H
C-19	20.0	11α-H, 19_b-H, 20-CH_3
C-20	12.7	11α-H, 19_a-H, 20-CH_3
C-21	82.4	4β-H, 21β-H, 23β-H
C-22	34.1	21β-H, 22α-H, 22β-H, 23β-H, 26-CH_3
C-23	34.7	22α-H, 22β-H, 23β-H, 26-CH_3
C-24	179.1	21β-H, 22α-H, 23β-H
C-26	14.8	22β-H, 23β-H

be better than 600 msec. The peaks a–f are quite useful for the elucidation of the stereochemistry. For example, the cross peak a,b indicates the proximity of the protons 11α-H and 1α-H to the olefinic proton 13-H; the cross peak c shows proximity of the proton 11α-H to 10α-H. Figure 6 shows the cross peak f indicating the close proximity of the proton 7α-H to the proton 11α-H.

We have carefully studied the molecular model of stemoninine. The assignment of the ketal of stemoninine should be appropriate in structure **3** when the olefinic proton 13-H is in close proximity to proton 11α-H. That means that the relative configuration of the substituents attached to C-12 was determined. We have not observed the cross peak between 2-H and 21-H in NOESY and all nuclear Overhauser effects in nOe-difference spectra, but the relative configurations of C-2 and C-23β have been determined in other *Stemona* alkaloids (4–9) to be 2α-H and 23β-H. The stereochemistry of stemoninine is, therefore, suggested as **3**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were determined in $CDCl_3$ with TMS as internal standard on a Bruker WH 400 spectrometer at 400 MHz and 100.6 MHz, respectively. All the 2D nmr spectra were recorded on a Bruker WH 400 and 200 spectrometer. ^{1}H shift-correlated 2D nmr spectra were observed by using the pulse 90°-t_1-45°-t_2 (10). We used a 45° rather than a 90° pulse as mixing pulse without delay time (^{1}H, ^{1}H-COSY 45, Figure 1).

The ^{13}C-^{1}H shift-correlated 2D nmr spectrum was obtained by using the refocusing delay time of 6.3 msec and the relaxation delay time of 1 sec (^{13}C, ^{1}H-COSY, Figure 2). The ^{13}C-^{1}H long-range shift correlated 2D nmr spectrum with polarization transfer via J-coupling experiment has been carried out with the aid of a Bruker micro-program (11); fixed delays D_3 and D_4 were adjusted to give maximum polarization for J_{CH}=8.0 Hz (^{13}C, ^{1}H-COLOC, Figure 3). The long range shift correlated 2D nmr with delay time of 350 msec was observed by using the 90°-Δ-t_1-45°-Δ-t_2 sequence (12) (Figure 4). The 2D nOe spectrum was measured by using the pulse sequence 90°-t_1-90°-t_2. We have used 600 msec and 1000 msec for τ_m, and $CDCl_3$ and C_6D_6 as solvent (NOESY, Figures 5 and 6) (13).

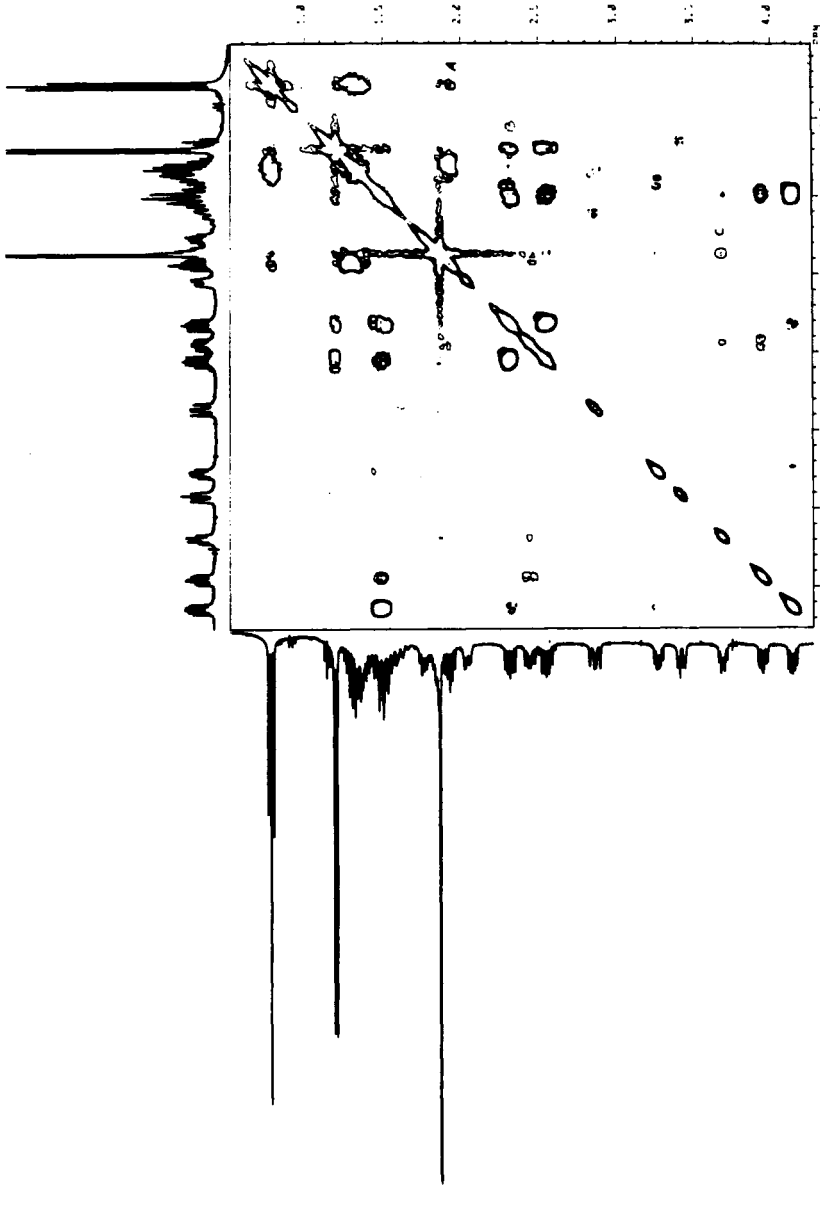

FIGURE 4. 2D long-range ^1H, ^1H shift-correlated spectrum of stemoninine [3] with delay time of 350 msec in $CDCl_3$, 400 MHz.

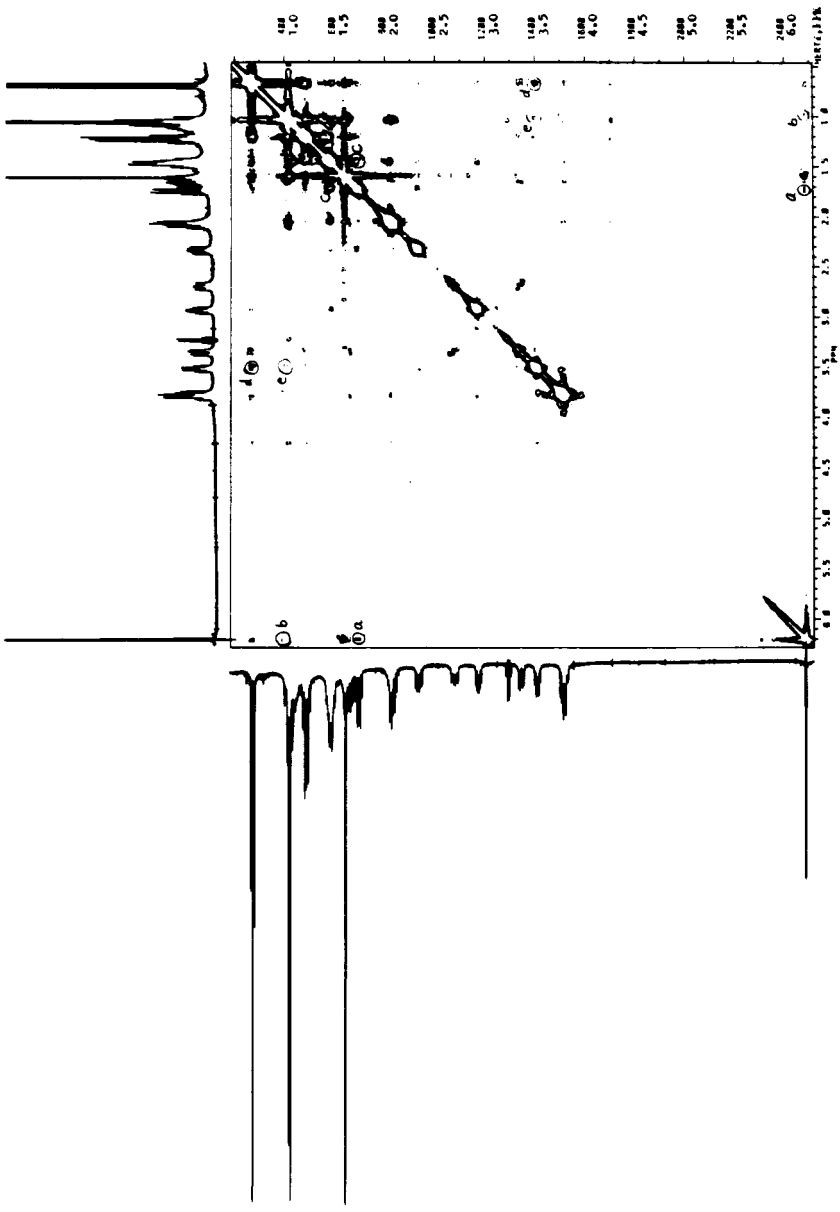

FIGURE 5. NOE-correlated 2D-nmr spectrum of stemoninine [3] with mixing time of 1000 msec in C_6D_6, 400 MHz.

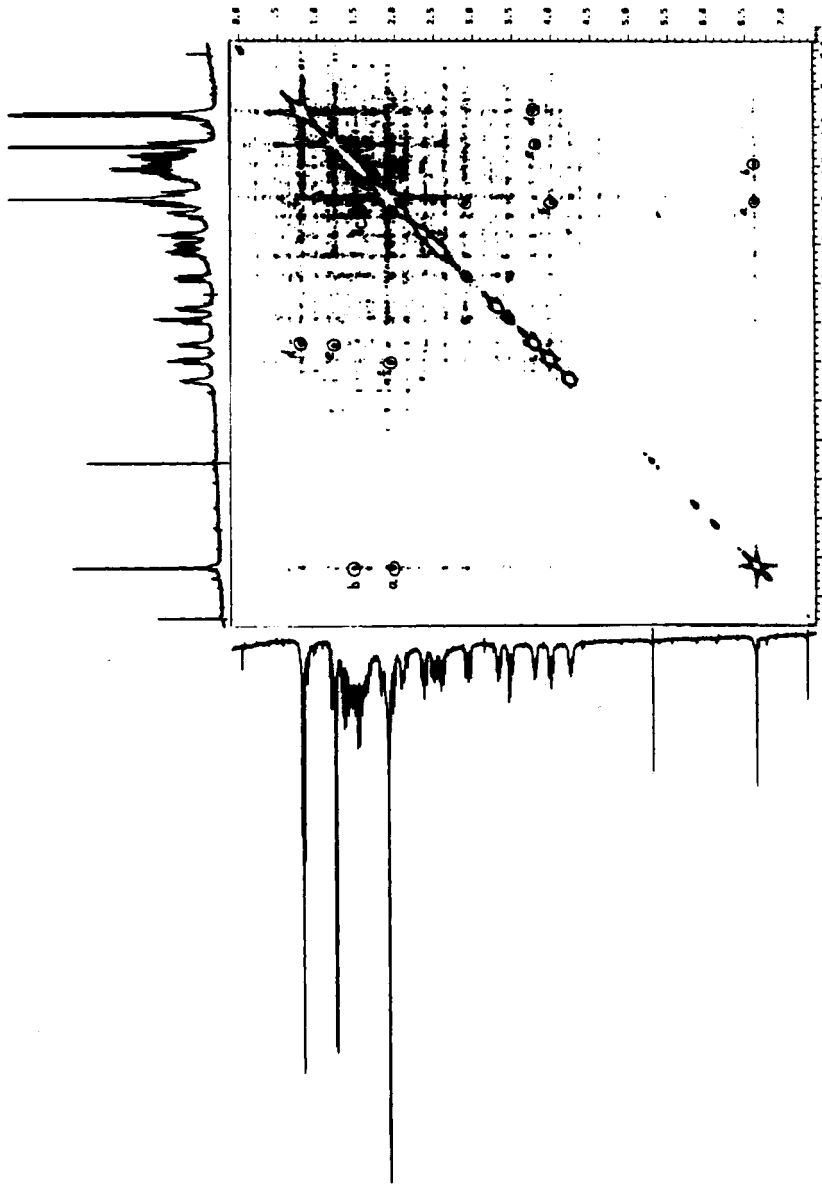

FIGURE 6. NOe-correlated 2D-nmr spectrum of stemoninine [3] with mixing time of 1000 msec in $CDCl_3$, 400 MHz.

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