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J. Nat. Prod., **1994**, 57 (5), 665-669• DOI:
10.1021/np50107a019 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 5, pp. 655-669, May 1994

ALKALOIDS OF *STEMONA JAPONICA*[1]

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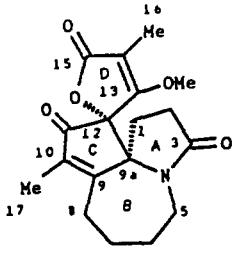
ABSTRACT.—Two new alkaloids, stemonamide [**1**] and isostemonamide [**2**], were isolated from the roots of a Chinese medicinal plant, *Stemona japonica*, collected from Zhejiang Province, People's Republic of China. Their structures were elucidated by spectral analyses and chemical conversion.

The roots of *Stemona japonica* (Stemonaceae) and related *Stemona* plants are used as insecticides and cough-relief agents. In our systematic investigation of alkaloids of Chinese *Stemona* plants, we have found almost all of the alkaloids present to have the same 4-aza-azulene basic ring skeleton. These structures can be separated into seven groups according to the sites of connection between the basic ring and the side-chain. In a previous publication (1), we have reported the presence of protostemonine-type alkaloids in this plant. The present study reports five maistemonine-type alkaloids in which maistemonine [**5**] could be regarded as a typical compound. According to a literature report, two alkaloids of this type, stemonamine and isostemonamine, have been isolated from the roots of *S. japonica* by Iizuka *et al.* (2). The structure of stemonamine [**3**] was determined by X-ray analysis and the structure of isostemonamine was also proposed as **4** (2). We have reported previously the structure of maistemonine and oxymaistemonine (3). On the basis of spectral comparison and chemical conversion, two new alkaloids, stemonamide [**1**] and isostemonamide [**2**], along with the known stemonamine [**3**], isostemonamine [**4**] and maistemonine [**5**] have been isolated and identified by spectral analysis and chemical correlation. The structures of maistemonine [**5**] and the related oxymaistemonine [**6**] have been corrected.

The molecular formula of stemonamide (**1**, $C_{18}H_{21}NO_5$) was obtained from hrms. The ir absorptions at 1755, 1715, and 1655 cm^{-1}, which were similar to those of stemonamine [**3**], indicated that the molecule contained unsaturated γ-lactone and ketone groups. A band at 1699 cm^{-1} was indicative of a lactam absorption. In comparison with the molecular formula and ^{13}C-nmr spectral data of stemonamine [**3**], **1** has one more oxygen and two less hydrogen atoms, and has a ^{13}C-nmr signal at δ 168.8 ppm instead of the common *N*-methylene carbon signal at about δ 50 ppm. A 45° ^1H-^1H COSY nmr spectrum showed the correlation between protons at C-1 and C-2, as well as between H-5 and H-8 on the seven-membered ring. The chemical shifts of geminal CH_2-5 (δ 4.18, br d, H-5α; δ 2.83, br t, H-5β) showed the influence of a carbonyl group affixed to C-3. A methoxy signal at δ 3.97 ppm and olefinic methyl signals at δ 2.00 (s) and δ 1.85 (s) indicated that compound **1** is a maistemonine-type alkaloid. Due to the small amount of the sample obtained from chromatography, chemical conversion was also carried out by oxidation of maistemonine [**5**] to **1** by $Pb(OAc)_4$. The oxidation product was identical to the natural isolate.

Isostemonamide [**2**] has the same molecular formula as **1**. Their ^1H- and ^{13}C-nmr spectra were closely comparable. Moreover, its ir absorptions at 1760, 1715, 1695, 1660, and 1635 cm^{-1}, and a series

[1]Part 6 in the series "Studies on *Stemona* Alkaloids." For part 5, see Ye and Xu (1).



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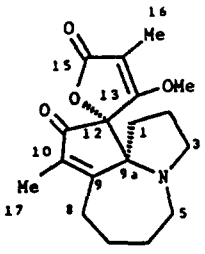

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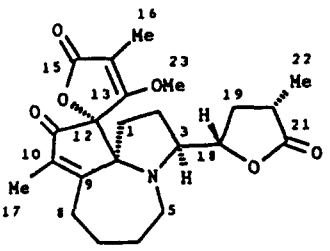

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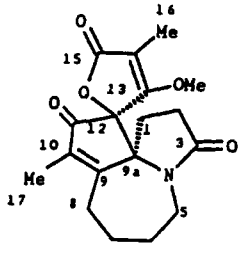

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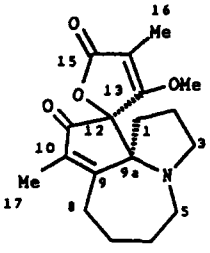

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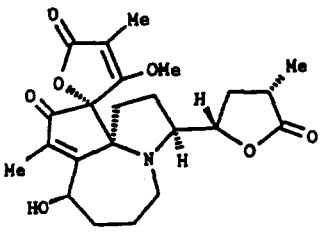

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of ^{13}C-nmr specific quaternary carbon signals (from C-9 to C-15) suggested it to be an isomer of **1**. The ROESY nmr correlations (Figure 1) between H-5β and H-7β, and H-5α and H-6α, H-6β, H-8β, and CH_3-17 indicated that the seven-membered ring was in a stable chair conformation. The cross-peak between H-8α and H-1α further indicated that the stereochemistry of the junctions between rings A, B, and C was the same as in stemonamine. In an nOe difference nmr spectral analysis of this series of compounds, irradiation of the methoxy group was found to lead to the enhancement of the C-16 methyl (ca. 4%). It is

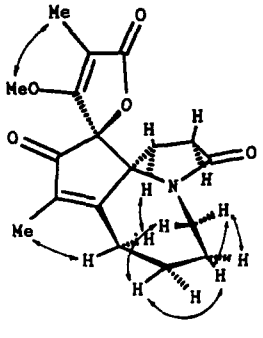

FIGURE 1. ROESY Nmr Correlations of Isostemonamide [**2**].

interesting to note that the spiro- orientation of ring D of these compounds can be detected by certain diagnostic ^{1}H- and ^{13}C-nmr signals (Table 1). Thus, alkaloids with the same ring-D orientation as stemonamine [**3**] have ^{1}H-nmr chemical shifts of the methoxy group at δ 3.96±0.01 ppm and of the C-16 methyl at δ 2.00±0.01 ppm, and the ^{13}C-nmr signals of C-12 at δ 90–92 ppm and the C-14 resonance at δ 96–100 ppm. In the isostemonamine [**4**] and isostemonamide [**2**] group, the chemical shifts of the methoxy and C-16 methyl were observed at δ 4.12±0.01 ppm and at δ 2.06 ppm, respectively; the ^{13}C-nmr signals of C-12 did not exceed δ 90 ppm and those of C-14 were over δ 102 ppm.

In support of these conclusions, maistemonine [**5**] was converted to an amide by $Pb(OAc)_4$ oxidation (4). The amide was demonstrated to be identical to natural stemonamide [**1**]. An nOe difference nmr spectral analysis of maistemonine was also carried out. As in the other cases, a 3.9% enhancement of the C-16 methyl signal was measured on irradiation of the methoxy group while no other proton signals responded to this irradiation. This nOe result ruled out the

TABLE 1. Comparison of Selected ^{1}H- and ^{13}C-nmr Data of Alkaloids **1–6** [δ (ppm), $CDCl_3$].

C,H	1	2	3	4	5	6
16-H	2.00	2.06	2.00	2.06	1.99	1.99
17-H	1.85	1.74	1.76	1.73	1.76	1.85
OMe-H	3.97	4.13	3.96	4.11	3.97	3.96
C-12	90.0	86.4	91.6	88.5	91.2	92.0
C-13	170.8	172.6	171.6	173.2	172.2	171.8
C-14	99.8	102.8	97.6	102.5	96.6	96.3
C-16	9.1	9.2	9.0	9.2	8.4	8.1
C-17	8.4	8.3	8.2	8.0	7.7	18.6

previous assumption of the spiro orientation of ring D which was mainly based on the NOESY cross-peaks between the methoxy and H-1α and H-2α (3). From a biosynthetic point-of-view, the structure of the related oxymaistemonine [**6**] previously reported by us (3) should also be corrected as shown in the present article.

Previous studies have shown that most of the *Stemona* alkaloids contain an α-methyl-γ-lactone ring annexed to C-3 of the pyrrolidine ring as a side-chain (5). Some of them, as in stemonamine [**3**] and stemonamide [**1**], have a methylene or carbonyl group instead. We can conclude after our systematic studies that all of the alkaloids can be separated into eight groups according to the sites of connections between the basic ring and the side-chain at C-9. In the maistemonine-type alkaloids, the connecting atom of this chain is C-9a, which bears the C-9a/C-12 bond and is connected to an α,β-unsaturated ketone ring and an α,β-unsaturated lactone ring, both of which are joined in a spiro-fashion at C-12. Because all of the atoms along the chain are quaternary carbons, it is difficult to determine the relative configuration of the α,β-unsaturated γ lactone ring (ring D). From Dreiding models, we have found that the lactone ring D is vertically oriented to ring C and the methoxy group is distant from all other functionalities except the C-16 methyl. Considering the biogenetic relationships and the ROESY nmr spectral results for **2**, we have concluded that the configurations of rings A, B, and C of this type of alkaloid are the same as in stemonamine as well as in other *Stemona* alkaloids. The cd and ord spectra of these compounds have been measured, but few standards are available to provide valuable information for structural determination.

Iizuka *et al.* (2) have proposed that **3** and **4** are interconvertable through an intermediate. In neither *Stemona japonica* nor *S. mairei* have we found the isomer of the major alkaloid, maistemonine [**5**]. We have concluded that these two groups of compounds are both naturally occurring and are not extraction artifacts.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler mp apparatus and are uncorrected. The ir spectra were recorded on a Perkin-Elmer 559B spectrophotometer. Hrms and eims data were collected on a MAT-711 spectrometer. ^{1}H-Nmr, ^{13}C-nmr, and 2D homo- and heteronuclear correlated nmr spectra were recorded on a Bruker AM-400, an AM-100, and a Varian GEM 300 spectrometer. The ROESY nmr spectrum was carried out on a Bruker AMX-600 spectrometer (in $CDCl_3$, with TMS as internal standard). Si gel was used for flash chromatography and was produced by Qindao Marine Chemical Industrials. The following solvent systems were applied for Si gel tlc: petroleum ether-Me_2CO (2:1), CH_2Cl_2-MeOH (95:5), and *n*-hexane-Me_2CO (1:1), and the compounds were detected by spraying with Dragendorff's reagent.

PLANT MATERIAL.—The roots of *Stemona japonica* (Blume) Miq. (Stemonaceae) were obtained from Anji County, Zhejiang Province, People's Republic of China, in autumn 1989. A voucher specimen is deposited in the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

TABLE 2. ^{13}C-nmr Data of Alkaloids **1–4** [δ (ppm), $CDCl_3$].

C	1[a]	2[a]	3[b]	4[b]
1	29.8	29.4	38.8	35.4
2	31.9	29.7	26.8	27.7
3	168.6	168.7	51.5	51.0
5	41.3	42.4	49.0	49.2
6	27.5	27.7	24.6	24.2
7	27.4	26.8	24.4	24.2
8	30.2	27.9	29.6	27.3
9	170.8	171.7	174.7	176.0
9a	74.6	73.5	77.4	75.4
10	137.0	136.6	135.2	134.6
11	196.5	196.9	198.4	—[c]
12	90.0	86.4	91.6	88.5
13	170.8	172.6	171.6	173.2
14	99.8	102.8	97.6	102.5
15	175.7	174.6	174.7	176.0
16	9.1	9.2	9.0	9.2
17	8.4	8.3	8.2	8.0
18	59.2	59.8	58.7	59.3

[a]Measured on a Varian GEM 300.
[b]Measured on a Bruker AM 100.
[c]This signal did not appear in the ^{13}C-nmr spectrum.

EXTRACTION AND ISOLATION.—The same extraction and fractionation procedures as previously described (1) were employed in the current study. Repeated chromatography carried out on Si gel for the first three alkaloid fractions [petroleum ether-Me_2CO (4:1)] yielded stemonamine [**3**] (60 mg) isostemonamine [**4**] (10 mg), and maistemonine [**5**] (81 mg). Further chromatography on Si gel and tlc of the petroleum ether-Me_2CO (1:1) fraction and prep. hplc gave stemonamide [**1**] (4 mg) and isostemonamide [**2**] (14 mg).

Stemonamide [**1**].—Mp 182.5–184°; $[\alpha]_D$ −120° (*c*=0.79, EtOH); ir ν max (KBr) 2920, 1755, 1715, 1699, 1655, 1630, 1385, 1125, 1000 cm^{-1}; eims *m/z* 331 $[M]^+$, 237, 224, 206, 165, 122; hrms *m/z* 331.1431 $[M]^+$, calcd for $C_{18}H_{21}NO_5$ 331.1418; ^{1}H- and ^{13}C nmr, see Tables 2 and 3.

Isostemonamide [**2**].—Mp 234–236°; $[\alpha]_D$ −177° (*c*=0.37, EtOH); ir ν max (KBr) 2960, 1760, 1715, 1695, 1660, 1635, 1390, 1340, 1120, 1010 cm^{-1}; eims *m/z* 331 $[M]^+$, 286, 270, 255, 204, 188, 83, 55; hrms *m/z* 331.1418 $[M]^+$, calcd for $C_{18}H_{21}NO_5$ 331.1418; ^{1}H and ^{13}C nmr are presented in Tables 2 and 3.

Stemonamine [**3**].—Mp 169–171°. Its physi-

TABLE 3. ^{1}H-Nmr Data of Alkaloids **1–4** [δ (ppm), $CDCl_3$].

H	1	2	3	4
1	α, 1.95, ddd	α, 1.90, ddd, 9.2, 12.8, 13.2	1.85, m	1.73, m
	β, 2.59, ddd	β, 2.59, dd, 13.2, 7.2	1.75, m	1.52, ddd
2	α, 2.28, dd, 14.6, 7.9	α, 2.26, ddd	1.85, m	2.34, dd, 5.7, 12.7
	β, 2.37, dd, 14.6, 8.8	β, 2.32, ddd, 9.2, 16.4, 12.8	1.85, m	1.73, m
3	—	—	3.05, ddd	2.82, dd, 13.5, 6.3
			3.10, m	3.17, m
5	α, 4.18, br d, 14.6	α, 4.14, br d, 10.8	2.75, m	3.17, m
	β, 2.83, br t, 12.9	β, 2.95, m	3.10, m	3.08, dd, 2.7, 15.6
6	α, 1.40, m	α, 1.36, m	2.10, m	1.75, m
	β, 1.82, br d, 10.5	β, 1.77, br d, 10.5	1.39, m	1.36, bdd, 14.3, 3.2
7	α, 2.13, m	α, 2.10, m	1.85, m	1.98, m
	β, 1.32, m	β, 1.26, m	1.20, ddd	1.13, m
8	α, 2.14, m	α, 2.10, m	2.87, ddd	2.81, m
	β, 2.98, dd, 5.7, 13.0	β, 2.93, dd, 12.2, 5.6	2.10, m	1.98, m
16	2.00, s	2.06, s	2.00, s	2.06, s
17	1.85, s	1.74, s	1.76, s	1.73, s
18	3.97, s	4.13, s	3.96, s	4.11, s

cal and spectral data were identical to those reported in the literature (2).

Isostemonamine [**4**].—Mp 155–157°. Its physical and spectral data were identical to those reported in the literature (2). ^1H and ^{13}C nmr are presented in Tables 2 and 3.

Maistemonine [**5**].—Mp 205–207°; ir, eims, ^1H- and ^{13}C-nmr data are the same as reported previously (4).

$Pb(OAc)_4$ OXIDATION OF MAISTEMONINE [**5**].—Because maistemonine did not react with $KMnO_4$ at room temperature, $Pb(OAc)_4$ was used in its oxidation (3). Maistemonine (100 mg) and $PbOAc_4$ (400 mg; wet) were mixed in 10 ml of THF, and stirred overnight at room temperature. The PbS obtained after introducing H_2S to the reaction mixture was filtered. Stemonamide [**1**] (18 mg) was obtained by prep. tlc, and the mmp, ir, and ^1H-nmr spectral data were identical to those obtained for **1**.

ACKNOWLEDGMENTS

This study was supported by The National Natural Science Foundation of China, Grant No. 28770103. We are also grateful to Mr. Kai Ma of the Shanghai Institute of Organic Chemistry for the ROESY nmr spectrum of compound **2**.

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Received 29 November 1993