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New Pyrrolo[1,2-*a*]azepine Type Alkaloids from *Stemona* and *Stichoneuron* (Stemonaceae)

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Summary. Three new pyrroloazepine type alkaloids, stichoneurines A and B and 6-hydroxycroomine were isolated from the lipophilic root extracts of *Stichoneuron caudatum* and *Stemona tuberosa* collected in Thailand together with the already known croomine, tuberostemonine, and tuberostemonine A. The structures were elucidated by spectroscopic methods including H/H-COSY, HMQC, and HMBC. Information on the relative stereochemistries and conformational behaviour was obtained by analysis of the N*O*ESY spectra. The formation of pyrroloazepine alkaloids in the genus *Stichoneuron* is reported for the first time and supports its affiliation to the family Stemonaceae. The occurrence of two different types of alkaloids, of the tuberostemonine and croomine series, in different geographical provenances of *Stemona tuberosa* is of special chemosystematic interest and may contribute to a more natural species delimitation within that complex group.

Keywords. Stichoneurines A-B; Tuberostemonine A; 6-Hydroxycroomine; Pyrroloazepine alkaloids; *Stichoneuron caudatum*; *Stemona tuberosa*.

Introduction

The formation of pyrrolo(1,2-*a*)azepine type alkaloids represents a characteristic chemical feature of the small monocotyledonous family Stemonaceae consisting of the three genera *Stemona*, *Croomia*, and *Stichoneuron*, comprising about 30 species. In a previous review 42 derivatives were listed mostly isolated from the roots of the largest genus *Stemona* [1]. Only one derivative, croomine (**5**), was reported from *Croomia* [2], whereas from *Stichoneuron* no chemical data were available so far.

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The chemical interest in *Stemona* extracts was stimulated by their popular use in southeast Asia as insecticides and vermifuges as well as against respiratory diseases [1, 3–6].

Structurally, *Stemona* alkaloids are characterized by a pyrrolo[1,2-*a*]azepine nucleus usually linked with two carbon chains mostly forming terminal lactone rings. Previously they were separated either into eight groups by *Ye et al*. [7] or five by *Pilli* and *Ferreira de Oliveira* [1]. However, in the meantime 76 derivatives have already been reported suggesting a new classification. Based on biosynthetic considerations and their various distribution in different *Stemona* species they can now be grouped into three skeletal types [8].

To complete the overview on the biosynthetic capacity of the family Stemonaceae we now investigated for the first time the underground parts (including roots and rhizomes) of the rare *Stichoneuron caudatum Ridley*, collected in south Thailand. From the methanolic extract we isolated a pair of two new isomeric alkaloids named stichoneurines A (**1**) and B (**2**). In this connection we also investigated extracts of two collections of *Stemona tuberosa Lour.*, originating from different habitats in southeast and north Thailand, showing completely different alkaloid profiles among each other. In contrast to the dominating tuberostemonine (**3**) in the southeastern habitat (HG 851), the collection from the northern habitat (HG 890) clearly deviated by an accumulation of croomine (**5**), representing a different skeletal type [1, 8]. Both collections contained two further alkaloids, **4** and **6**, which showed close structural affinities to tuberostemonine and croomine. Apart from the well-known tuberostemonine (**3**) in collection HG 851, already previously described for the southeastern habitat [4], we now also isolated its stereoisomer tuberostemonine A (**4**) so far only known from its first description in *Stemona sessilifolia Miq*. [9]. In addition to croomine (**5**), the northern collection (HG 890) also contained 6-hydroxycroomine (**6**), which was shown to be an as yet unknown compound. In the present paper we report the isolation and structure elucidation of the three new alkaloids **1**, **2**, and **6**, and discuss the NMR data of tuberostemonine A (**4**), which were not available in literature. The NMR data of tuberostemonine (**3**), so far only documented as supporting information for a synthetic product [10], were also included in the discussion.

Results and Discussion

The $CHCl_3$ fractions of the methanolic root extracts of *Stichoneuron caudatum* and two different geographical provenances of *Stemona tuberosa* were compared. Since all major fractions did not show characteristic UV absorptions they were detected with *Dragendorff* reagent and isolated by preparative TLC. The IR spectra of the new compounds were very similar in CCl_4 showing very strong absorption bands between 1778–1781 cm^{-1} indicative for the carbonyl groups of lactone rings and two characteristic signals between 1156–1187 cm^{-1} and 1013–1016 cm^{-1}. The alkaloid fraction detected in *Stichoneuron* turned out to be a mixture of two inseparable stereoisomers (**1**, **2**) whose structures were elucidated unambiguously by NMR experiments. In the ^{13}C NMR a complete twofold set of lines in the intensity ratio of 60:40 was shown and some resonances appeared also twice in the ^{1}H NMR spectrum. Signals with large diastereomeric splittings were observed for the

^{1}H resonances 9, 11, and 16 (0.07–0.10 ppm) and 9a, 10, and 12 in the ^{13}C NMR (>1 ppm). This suggested some different configuration(s) in this region of the molecule. Due to the clear intensity differences of the ^{13}C NMR resonances for components **1** and **2** and the use of 2D methods (COSY, HMQC, HMBC, and N*O*ESY) it was possible to assign all ^{13}C resonances of both isomers. Both were characterised by the presence of 3 methyl groups, 9 methylenes, 8 methines, and two quaternary carbon atoms. The molecular formula $C_{22}H_{35}NO_4$ for **1** and **2** was confirmed by HREIMS ($m/z = 377.2573$). The very dominant base peak at $m/z = 278$ corresponds to a loss of 99 which is common for many *Stemona* alkaloids and was interpreted as the loss of a five membered lactone ring $C_5H_7O_2$ at C-3 of the pyrroloazepine moiety. The ^{1}H NMR spectrum showed the presence of two methine protons next to lactonic ring oxygens. One appeared as a pair of two well separated ddd at $\delta = 4.63/4.56$ ppm with an integration 61/39 (±2%) (11-H), the other one at $\delta = 4.17$ ppm showed no diastereomeric splitting (18-H) (Table 1). Additionally, the ^{1}H NMR showed two methyl dublets at $\delta = 1.28/1.30$ ppm (15-H_3) and 1.26/1.26 (22-H_3, no diastereomeric splitting), both characteristic for the lactone rings in *Stemona* alkaloids. The two methyl substituted lactone rings and the molecular formula as well as the ^{1}H methyl triplet at $\delta = 0.97/0.95$ ppm for an ethyl group agreed with the general structure of tuberostemonine (**3**). However, two additional hydrogens were found in **1** and **2**. Moreover, in relation to tuberostemonine two methines have been changed to two methylenes and one of the rings had been opened by C–C cleavage. The H/H-COSY cross peaks resulted in connectivities 15–13–12–11–10–16–17 with a branching 10–9–9a, and also clearly showed the sequences 22–20–19–18–3–2 and 5–6–7. Long range HMBC contacts C-9a → 5-H_2 and C-3 → 5-H_2 close the perhydropyrroloazepine system. All assignments are compiled in Table 1. For the overlapping methylenes nos. 1, 2, 6, 7, and 8 no detailed analyses were possible. The stereochemistry was derived from the characteristic N*O*ESY cross peak 11 ↔ 15 and the N*O*ESY contacts 9 ↔ 9a, 9a ↔ 18, 18 ↔ 20, which were all common to both compounds **1** and **2**. However, one striking difference between the two isomers was shown in the N*O*ESY spectrum. On the one hand cross peaks of the larger 60% resonance of 9-H ($\delta = 2.03$ ppm) and 9a-H ($\delta = 3.41$ ppm) with the larger 17-H_3 triplet ($\delta = 0.97$ ppm) of the ethyl group at C-10 of **1** could be observed and, on the other hand, a strong cross peak of the smaller 40% resonance of 11-H ($\delta = 4.56$ ppm) with the smaller 17-H_3 resonance ($\delta = 0.95$ ppm) of **2** was found. This strong 17-H_3 ↔ 9-H interaction for **1** and the strong 17-H_3 ↔ 11-H contact for **2** is conclusive that the two diastereomers differ by the configuration of the C-10 ethyl group. Important N*O*ESY correlations are indicated in the formulae for **1** and **2**. The new compounds were designated as stichoneurine A (**1**) and stichoneurine B (**2**).

Analysis of the configurations showed that **2** is closely related to tuberostemonine B [11]. These structures can be transformed into each other by ring closure between C-1 and C-12 of stichoneurine B (**2**) or *vice versa*, by C-1–C-12 ring opening of tuberostemonine B. Due to biosynthetic relationships in this series of alkaloids, the absolute configurations can be inferred from the known stereochemistry of tuberostemonine determined by X-ray analysis [12]. Stichoneurine A (**1**) has not yet a known counterpart in the tuberostemonine series. A similar tuberostemonine related alkaloid with the same substituent at C-9, containing the characteristic

Table 1. ^{1}H and ^{13}C NMR data (δ/ppm) for **1**–**4** and **6** (400 MHz, $CDCl_3$)[a, b]

	^{1}H NMR				
	1 (60%)	**2** (40%)	**3**	**4**	**6**
1	a: ~1.60 m		1.80 m	1.81 m	a: 1.92 m
	b: ~1.45 m				b: 1.54 m
2	a: ~1.90 m		a: 2.18 ddd	a: 1.84 m	a: 1.95 m
	b: ~1.40 m		b: 1.10 m	b: 1.60 m	b: 1.46 m
3	3.34 m	3.34 m	3.43 m	3.09 ddd	3.42 ddd
5	a: 3.39 m	a: 3.39 m	a: 3.47 ddd	a: 2.98 ddd	a: 3.53 dd
	b: 2.88 ddd	b: 2.88 ddd	b: 2.67 ddd	b: 2.44 ddd	b: 3.07 dd
6	a: ~1.70 m		a: 1.53 m	a: 1.68 m	3.87 br.m
	b: ~1.60 m		b: 1.44 m	b: 1.47 m	
7	a: ~1.80 m		a: 1.84 m	a: 1.93 m	a: 2.01 m
	b: ~1.20 m		b: 1.17 m	b: 1.22 m	b: 1.71 m
8	a: ~1.60 m		a: 1.57 m	a: 1.65 m	a: 2.05 m
	b: ~1.45 m		b: 1.54 m	b: 1.40 m	b: 1.77 m
9	2.03 m	1.93 m	1.82 m	1.57 m	
9a	3.41 m	3.41 m	3.07 dd	2.63 dd	3.21 dd
10	1.40 m	1.35 m	1.55 m	1.42 m	a: 2.47 dd
					b: 1.74 m
11	4.63 ddd	4.56 ddd	4.44 dd	4.44 dd	2.71 m
12	a: 2.21 m	a: 2.18 m	2.00 ddd	2.01 ddd	
	b: 1.96 m	b: 1.91 m			
13	2.64 m	2.69 m	2.41 dq	2.33 dq	1.32 d
14					4.39 ddd
15	1.28 d	1.30 d	1.28 d	1.26 d	a: 2.39 ddd
					b: 1.44 m
16	a,b: 1.43 m	a,b: 1.35 m	a: 1.52 m	a: 1.64 m	2.61 m
			b: 1.50 m	b: 1.55 m	
17	0.97 t	0.95 t	0.96 t	0.91 t	
18	4.17 ddd	4.17 ddd	4.31 ddd	4.31 ddd	1.27 d
19	a: 2.36 m	a: 2.35 m	a: 2.38 ddd	a: 2.37 m	
	b: 1.55 m	b: 1.55 m	b: 1.47 m	b: 1.90 m	
20	2.61 m	2.59 m	2.60 ddq	2.69 ddq	
21					
22	1.26 d	1.26 d	1.26 d	1.30 d	
	^{13}C NMR				
	1	**2**	**3**	**4**	**6**
1	26.33 t*	25.62 t*	41.6 d	41.4 d	27.6 t
2	27.09 t	26.95 t	32.1 t	31.6 t	25.1 t
3	64.21 d	63.87 d	65.0 d	65.1 d	68.3 d
5	47.79 t	47.15 t	48.1 t	53.3 t	53.4 t
6	27.93 t	27.07 t	28.1 t	26.0 t	68.4 d
7	29.38 t	29.43 t	29.9 t	29.9 t	29.9 t
8	25.76 t*	25.44 t*	30.4 t	29.9 t	33.8 t
9	42.08 d	41.86 d	40.7 d	40.6 d	88.1 s

(*continued*)

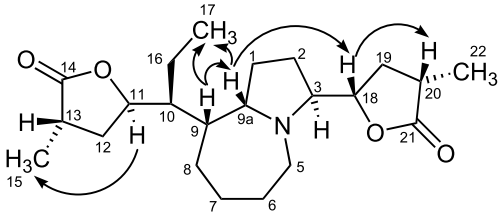
Table 1 (*continued*)

	^{13}C NMR				
	1	**2**	**3**	**4**	**6**
9a	64.80 d	66.13 d	63.6 d	68.3 d	68.9 d
10	49.09 d	47.36 d	45.0 d	42.5 d	38.4 t
11	80.25 d	80.20 d	80.3 d	80.2 d	35.9 d
12	34.92 t	33.05 t	47.3 d	46.9 d	178.9 s
13	34.51 d	34.78 d	40.9 d	40.1 d	17.5 q
14	180.30 s	180.39 s	179.2 s*	179.2 s*	78.6 d
15	16.49 q	16.66 q	14.7 q	15.2 q	35.2 t
16	21.07 t	20.24 t	24.3 t	22.4 t	34.8 d
17	13.91 q	14.10 q	11.2 q	9.8 q	179.0 s
18	83.60 d	83.57 d	81.4 d	80.8 d	14.9 q
19	34.25 t	34.19 t	34.6 t	32.5 t	
20	35.01 d	34.99 d	34.8 d	35.6 d	
21	179.60 s	179.64 s	179.4 s*	179.4 s*	
22	14.96 q	14.96 q	14.9 q	15.2 q	

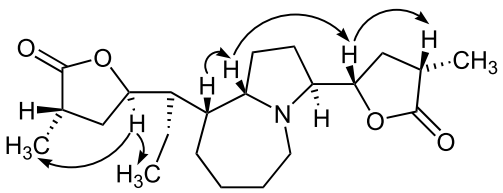
[a] Coupling constants: **1** + **2**: 5_b (15.5, 11.4, 4.2 Hz) (**1** and **2**), 11 (7.4, 7.4, 4.1 Hz) (**1**) and (7.1, 7.1, 5.3 Hz) (**2**), 15 (7.4 Hz) (**1** and **2**), 18 (10.6, 7.1, 5.3 Hz) (**1** and **2**), 17 (7.1 Hz) (**1** and **2**), 22 (7.0 Hz) (**1** and **2**); **3**: 2_a (12.3, 6.7, 6.7 Hz), 5_a (14.4, 2.8, 1.4 Hz), 5_b (14.4, 10.6, ~1 Hz), 9a (11.1, 6.3 Hz), 11 (7.8, 7.5 Hz), 12 (10.4, 7.5, 7.3 Hz), 13 (7.3, 7.3 Hz), 15 (7.3 Hz), 17 (7.4 Hz), 18 (10.4, 7.6, 5.7 Hz), 19_a (12.4, 8.5, 5.7 Hz), 20 (12.4, 8.5, 7.0 Hz), 22 (7.0 Hz); **4**: 3 (10.4, 3.5, 2.8 Hz), 5_a (12.5, 4.2, 3.8 Hz), 5_b (12.5, 10.8, 2.4 Hz), 9a (10.6, 8.8 Hz), 11 (12.1, 9.7 Hz), 12 (9.7, 9.7, 9.7 Hz), 13 (9.7, 7.3 Hz), 15 (7.3 Hz), 17 (7.4 Hz), 18 (10.4, 5.6, 3.5 Hz), 20 (12.3, 8.5, 7.0 Hz), 22 (7.0 Hz); **6**: 3 (8.2, 7.1, 4.2 Hz), 5_a (14.1, 4.3 Hz), 5_b (14.1, 1.3 Hz), 6 ($w_{1/2}$ 13 Hz), 9a (8.2, 7.0 Hz), 10_a (13.5, 10.3 Hz), 13 (7.3 Hz), 14 (10.7, 8.2, 5.5 Hz), 15_a (12.5, 8.3, 5.5 Hz), 18 (7.1 Hz) , 6-OH 2.65 v.br.m, $w_{1/2}$(OH) = ~40 Hz;
[b] interchangeable within the column (*)

lactone ring and the ethyl group at C-10, was recently described for *Stemona sessilifolia* [13]. However, in these sessilifoliamides the lactone ring at C-3 was most probably removed by oxidation to a 3-carbonyl group, leading to a lactamic ring. The configurations at C-11 and C-13 were also different to those of stichoneurines A or B.

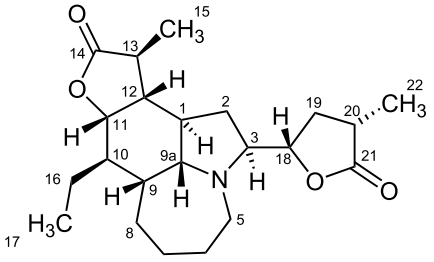
The two collections of *Stemona tuberosa*, HG 851 and HG 890, can be clearly distinguished chemically by different alkaloid profiles consisting of the two major compounds **3** and **4**, or **5** and **6**. The NMR spectra of **3** as well as the EIMS with $m/z = 375$ (M^+) and a 100% peak at $m/z = 276$ (M-99) together with a m.p. 86–87°C suggested the structure of tuberostemonine (**3**) (m.p. 86–88°C in Ref. [9]). Tuberostemonine is very often cited in literature, however, to our knowledge reliable NMR data are limited to a figure of a low resolution ^{1}H NMR of the natural product without further comments [9] and the ^{1}H and ^{13}C data of a synthetic product in CD_2Cl_2 as solvent [10]. Therefore we have analysed the ^{1}H and ^{13}C NMR of tuberostemonine (**3**) by 2D techniques and included all assignments in Table 1. In the H/H COSY spectrum all expected connectivities were found, only the resonances of 10-H and 16-H_2 were too close to each other to give clear cross peaks. The assignments of all ^{1}H and ^{13}C resonances were completed by means of



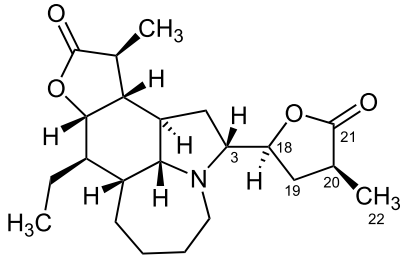
1 Stichoneurine A



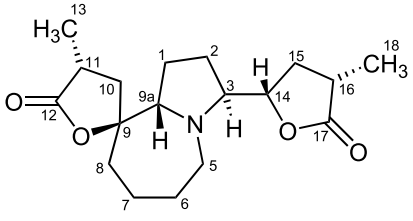
2 Stichoneurine B



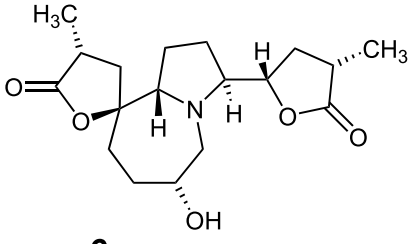
3 Tuberostemonine



4 Tuberostemonine A



5 Croomine



6 6-Hydroxycroomine

Formulae

HMQC and HMBC. Characteristic N*O*ESY contacts were in full agreement with the known stereochemistry of **3**. Some decisive cross peaks for β positions are *e.g.* from 11-H to 12-H, 9-H, 9a-H, 15-H_3, 16-H_2, and 17-H_3 and also from 18-H to 20-H and 9a-H. Additionally the α positions are linked by the N*O*ESY cross peak 1-H $\leftrightarrow$ 3-H.

Besides the dominating tuberostemonine (**3**) a second less polar alkaloid (**4**) was isolated from HG 851, whose mass spectrum was nearly identical with that of **3**. HREIMS for **4** ($m/z = 375.2416$) confirmed the molecular formula $C_{22}H_{37}NO_4$ which matched that of tuberostemonine (**3**) and its numerous isomers already known so far [8]. The most important fragmentation, the loss of the lactone ring at C-3 at $m/z = 276$ (M-99), was also in favour of this general structure and the ^1H and ^{13}C NMR spectra of **4** agreed perfectly with a tuberostemonine type structure and showed all significant resonances, *e.g.* the low field resonances of 11-H and 18-H of the two lactone rings as well as the three methyl resonances 15-, 22-, and 17-H_3 (two dublets and one triplett). Since the number of methyls, methylenes,

methines, and quaternary carbon atoms in the ^{13}C NMR agreed as well, we expected a stereoisomer of tuberostemonine (**3**). However, a comparison of the available ^{13}C NMR data for tuberostemonine B and C [11], tuberostemonine J and H [6], and neotuberostemonine [14] did not fit our data. Therefore a thorough structure elucidation of compound **4** was necessary.

Evaluation of the COSY cross peaks of **4** resulted in the bond sequences 15–13–12 (branching to 1) –11–10, 17–16, 22–20–19–18–3–2, 9–9a–1, and 5–6. The resonances for 1, 2, 6, 7, 9, 10, and 16 are too close for a clear identification of the remaining connectivities. However, by means of C/H (HMQC) and long range correlation (HMBC) all ^1H and ^{13}C NMR resonances could be identified (Table 1). The HMBC contacts across the nitrogen atom C-3 $\leftrightarrow$ 5-H_2 and C-9a $\leftrightarrow$ 5-H_2 are typical for the nitrogen fused perhydropyrroloazepine system, the assignments of the quaternary lactone carbonyls C-14 and C-21 followed from the long range interactions C-14 $\leftrightarrow$ 11-H, C-14 $\leftrightarrow$ 15-H_3, and C-21 $\leftrightarrow$ 22-H_3. Information on the stereochemistry and also on the conformation of **4** followed clearly from N*O*ESY correlations. The strong N*O*ESY interaction between the diastereomeric protons 5-Ha $\leftrightarrow$ 19-Hb is only possible when the conformation of the lactone ring is different to that usually depicted for the tuberostemonines (*e.g.* **3**). Obviously the lactone ring has been rotated about the C-3 – C-18 bond due to stereochemical requirements. The reason is a change of the configuration at C-3, which is 3-H_α in tuberostemonine (**3**), but 3-H_β in compound **4**. This followed unambiguously from the N*O*ESY contacts of resonances in β positions: 3-H $\leftrightarrow$ 9a-H, 9a-H $\leftrightarrow$ 11-H, 9a-H $\leftrightarrow$ 12-H, 11-H $\leftrightarrow$ 12-H, 12-H $\leftrightarrow$ 15-H_3, and 11-H $\leftrightarrow$ 17-H_3. This means that all configurations at positions 9, 9a, 10, 11, 12, and 13 are identical in tuberostemonine (**3**) and compound **4**, only the conformation at position 3 is opposite. In this case the lactone ring of **4** adopts again a conformation with 3-H and 18-H in a transoid arrangement, resulting in a sterically favorable transoid arrangement of the bulkier substituents at C-3 and C-18. The N*O*ESY cross peak of one of the diastereomeric 5-H_2 protons, 5-H_b $\leftrightarrow$ 9a-H, proved the β position of 5-H_b and the cross peak between the α protons 5-H_a $\leftrightarrow$ 18-H was again in perfect agreement with the conformational principles outlined above. The centers of asymmetry in the lactone ring itself (C-18 and C-20) are equally configurated in tuberostemonine and in compound **4**. This follows from N*O*ESY contacts of the α-protons 18-H $\leftrightarrow$ 20-H; due to the rotation of the complete ring about the C-3–C-18 bond this corresponds to β in the structural formula of tuberostemonine. Compound **4** was therefore identical with tuberostemonine A, where the only difference to tuberostemonine (**3**) is a different configuration at C-3. This compound was described only once as early as 1962 [9]. In that case the compound was isolated as a minor component from *Stemona sessilifolia* but no detailed NMR data were given at that time. It was simply stated that the 60 MHz ^1H NMR looked rather similar to that of tuberostemonine (**3**).

In contrast to these results the root extract of *Stemona tuberosa* originating from the northern habitat (HG 890) did not show any tuberostemonine derivatives. Instead, two other alkaloids were isolated from which the major derivative turned out to be the already known croomine (**5**). It was identified by comparison with ^1H and ^{13}C NMR data listed in the original description of *Noro et al.* [2] and in a further study on the ^1H NMR behaviour of *Stemona* alkaloids by *Xu et al.* [15]. The

NMR data of the second alkaloid (**6**) resembled those of croomine (**5**), but showed as main difference an additional low field CH group at $\delta = 3.87$ ppm (br.m) in the ^{1}H NMR and at $\delta = 68.4$ ppm (d) in the ^{13}C NMR spectrum. The EIMS of **6** showed an $m/z = 238$ (100% peak) obviously after the usual loss of the lactone ring at C-3. Indeed, FABMS gave a peak M + H of $m/z = 338$ and the high resolution gave 338.1952 which matches a molecular formula of $C_{22}H_{37}NO_4$ for **6**. ^{1}H NMR and ^{13}C NMR showed the typical methyl groups nos. 13 and 18 for two lactonic rings ($\delta = 1.32$ (d)/17.5 (q) ppm and 1.27 (d)/14.9 (q) ppm) and also the two lactonic carbonyls nos. 12 and 17 in the ^{13}C NMR spectrum ($\delta = 179.03$ and 178.94 ppm) (Table 1). However, only one low field methine no. 14 next to the lactone oxygen ($\delta = 4.39$ (ddd) 78.6 (d) ppm) was observed. The second corresponding carbon atom next to oxygen appeared as a singlet in the ^{13}C NMR spectrum at $\delta = 88.1$ ppm. This value was comparable to the chemical shift of the spiro carbon of croomine ($\delta = 89.3$ ppm) [2]. The molecular mass corresponded to an oxygenated croomine. Obviously a hydroxy group was attached to one of the methylene groups of croomine giving the additional low field CH group ($\delta = 3.87$ (br.m)/68.4 (d) ppm). Two further low field CH belonged to positions 3 and 9a next to the nitrogen atom of the pyrroloazepine core (Table 1). Strong H/H COSY cross peaks between the diastereomeric N–CH_2 protons (5-H_2) and the low field methine proton geminal to the hydroxy group ($\delta = 3.87$ (br.m) ppm) showed clearly that the hydroxy group was attached to C-6 of the azepine ring. In the H/H COSY spectrum all connectivities for 6-hydroxylated croomine could be found and in combination with HMQC and HMBC all resonances could be assigned unambiguously. The N*O*ESY cross peaks between 14-H ↔ 16-H, and 14-H ↔ 9a-H proved the configurations of the C-3 lactone ring relative to the decahydropyrroloazepine moiety and the cross peak 9a-H ↔ 11-H linked the stereochemistry of the spiro-lactone ring with the rest of the molecule. Only the observation of a rather low level cross section in the N*O*ESY spectrum revealed a further cross peak between 9a-H ↔ 6-H. This cross peak was very weak, however, it appeared identically on both sides of the diagonal of the 2D plot. This implies that the proton 6-H belongs to the β-network as well (11-H, 9a-H, 14-H, 16-H, 6-H) and the 6-OH group is therefore orientated in an α position. These relative configurations determined by N*O*ESY fit exactly to the absolute configurations determined by X-ray structure analysis of croomine methiodide [2]. Compound **6** was designated as 6-hydroxycroomine.

With respect to the restricted distribution of that type of pyrrolo[1,2-*a*]azepine alkaloids in the plant kingdom, only known so far from the genera *Stemona* and *Croomia* of the family Stemonaceae [1], their occurrence in the genus *Stichoneuron* deserves special chemosystematic interest. The formation of the characteristic C-10 ethyl group in stichoneurines A and B (**1**, **2**) showed a close structural relationship to the tuberostemonine group [8]. Since tuberostemonine (**3**) itself together with related derivatives represent a typical chemical character of the *Stemona tuberosa* group, its replacement by croomine (**5**) and the related derivative **6** with a different skeletal type was surprising and might contribute to a more natural grouping within that complex species group. At the same time, however, this finding supported the relationship between *Stemona* and *Croomia* [2]. Due to the chemical instability of some tuberostemonine derivatives [8, 16, 17] the possibility could not be excluded that tuberostemonine A (**4**) is actually an artifact, formed by

isomerisation of tuberostemonine (**3**). However, careful extraction from fresh plant material at room temperature as well as re-examination of **3** and **4**, which had been set aside for an extended period, suggested that tuberostemonine A (**4**) can be regarded as a naturally occurring alkaloid.

Experimental

NMR: Bruker DRX400 WB; MS: Finnigan MAT 900 S; IR: Perkin-Elmer 16PC FT-IR; Optical rotation: Perkin-Elmer polarimeter 241.

Stichoneuron caudatum was collected in south Thailand, Than To waterfall between Yala and Betong (HG 898), and *Stemona tuberosa* from two different habitats: (a) from southeast Thailand, Rayong, Khao Chamao (HG 851), and (b) from north Thailand, Chiang Mai, Kun Chang Kien (HG 890). Voucher specimens are deposited at the Herbarium of the Institute of Botany, University of Vienna (WU).

Extraction and Isolation

Dried underground parts (33 g, rhizomes and roots) of *Stichoneuron caudatum* (HG 898) were extracted with *Me*OH at room temperature for 4 d, filtered, and concentrated. The remaining aqueous phase was extracted with $CHCl_3$ (294 mg) and roughly separated on a silica gel column (Merck silica gel 60, 0.2–0.5 mm) by elution with *n*-hexane/Et_2O with Et_2O increasing from 0 to 100% and finally with 0–50% *Me*OH in Et_2O. The fractions with positive *Dragendorff* reaction, eluted with 25–50% *Me*OH in Et_2O, were combined (70 mg) and further separated by preparative TLC (CH_2Cl_2/*Et*OAc/*Me*OH/diethylamine = 70/30/5/1) leading to an alkaloid fraction (21 mg) from which 7 mg of the stereoisomeric mixture of stichoneurines A + B (**1** + **2**) was isolated by repeated TLC.

Dried roots (18 g) of *Stemona tuberosa* (HG 890) were extracted as above leading to 150 mg of lipophilic crude extract ($CHCl_3$). CC afforded 65 mg of alkaloid containing fractions which were further separated by preparative TLC yielding 11 mg **5** and 7 mg **6**.

Fresh roots (32 g) of *Stemona tuberosa* (HG 851) afforded 60 mg $CHCl_3$ extract from which 30 mg were further separated by preparative TLC leading to 8 mg **4** and 10 mg **3**. See also Ref. [4].

Stichoneurines A/B (**1**, **2**)

IR (CCl_4): $\bar{\nu} = 2967$ w, 2931 m, 1779 s, 1456 m, 1379 w, 1186 m, 1152 w, 1016 m, 939 w, 921 w cm^{-1}; MS (70 eV): $m/z = 377$ (M^+, 6), 278 ($M^+ - C_5H_7O_2$, 100), 234 (5), 149 (8), 55 (12); HREIMS: $m/z = 377.2573$, calcd for $C_{22}H_{35}NO_4$ 377.2566.

Tuberostemonine A (**4**)

$[\alpha]_D^{20} = -55^\circ\ cm^2\ g^{-1}$ ($CHCl_3$, $c = 0.4$); IR (CCl_4): $\bar{\nu} = 2968$ w, 2931 m, 1781 s, 1456 m, 1380 w, 1162 m, 1013 m, 925 w cm^{-1}; MS (70 eV): $m/z = 375$ (M^+, 5), 276 ($M^+ - C_5H_7O_2$, 100), 232 (4), 176 (4), 134 (5), 81 (5), 55 (8); HREIMS: $m/z = 375.2416$, calcd for $C_{22}H_{33}NO_4$ 375.2410.

6-Hydroxycroomine (**6**)

$[\alpha]_D^{20} = +23^\circ\ cm^2\ g^{-1}$ ($CHCl_3$, $c = 0.5$); IR (CCl_4): $\bar{\nu} = 2968$ w, 2931 m, 1779 s, 1456 w, 1379 w, 1187 m, 1156 m, 1016 m, 1010 m, 986 w, 931 w cm^{-1}; MS (70 eV): $m/z = 238$ ($M^+ - C_5H_7O_2$, 100), 210 (7), 140 (11); HRFABMS: $m/z = 338.1952$ ($M^+ \cdot H$), calcd for $C_{18}H_{27}NO_5 \cdot H$ 338.1967.

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