

## Structure Elucidation of the Binary Indole Alkaloid Uncaramine.

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The structure elucidation of uncaramine (1), a binary alkaloid derived from gambirine and pseudoyohimbine moieties linked together through a C(10)–C(21') bond, has been performed mainly using  $^1\text{H}$  and  $^{13}\text{C}$  n.m.r. spectroscopy. The presence of a hydrogen bond between the 9-hydroxy proton and the 4'-nitrogen atom established a preferred orientation of the two halves, the C(11)–C(10)–C(21')–H(21') dihedral angle being *ca.* 20°. A complete assignment for the  $^1\text{H}$  n.m.r. spectrum of gambirine (2) is also presented.

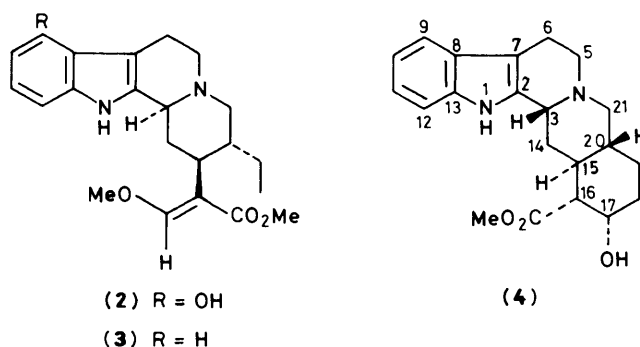
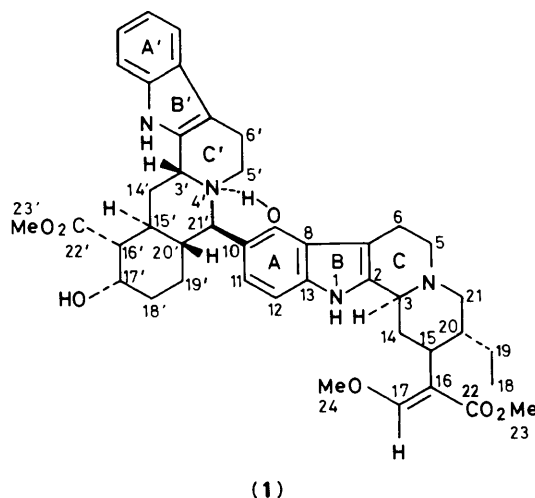
Extensive studies<sup>1–4</sup> in our laboratory on the constituents of Malaysian *Uncaria* species (family Rubiaceae) have resulted in the isolation of many new indole alkaloids; in particular, from *Uncaria gambier* Roxb. the indole alkaloids gambirine (2) and dihydrocorynantheine (3) together with some oxindole alkaloids have been isolated.<sup>2,5</sup> The chemotaxonomic significance of the distribution of the alkaloids among *Uncaria* sp. has been discussed after the isolation of alkaloids from all the known (400) species.<sup>6</sup>

The first isolation of a binary alkaloid, whose structure could not be elucidated, from the leaves of *Uncaria gambier* Roxb. was reported in 1972 by two of us.<sup>2</sup> Based on chemical and spectral data it was postulated that this compound was formed from disubstituted dihydrocorynantheine and yohimbine-type units. Most recently Goh *et al.*<sup>7</sup> obtained from *Uncaria callophylla* a compound exhibiting an  $^1\text{H}$  n.m.r. spectrum closely similar to that showed by the alkaloid (1), and whose structure again could not be determined. Based on u.v., i.r., and  $^1\text{H}$  n.m.r. data, they postulated however that it was probably formed from gambirine and pseudoyohimbine moieties linked *via* the 10- or 12-positions of gambirine and the 5'- or -21'-positions of pseudoyohimbine (4).

We report here the structure and the preferred conformation of the binary alkaloid (1), which we propose to call uncaramine, from  $^1\text{H}$  and  $^{13}\text{C}$  high-resolution n.m.r. results.

The high-resolution mass spectrum established the molecular formula of (1) as  $\text{C}_{43}\text{H}_{52}\text{N}_4\text{O}_7$  in full agreement with the previously reported hypothesis and showed the two complementary fragments  $\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_3$  and  $\text{C}_{22}\text{H}_{27}\text{N}_2\text{O}_4$ . In addition the intensities of four of the signals in the 300 MHz  $^1\text{H}$  n.m.r. spectrum of (1) in  $(\text{CD}_3)_2\text{SO}$  decreased upon addition of deuterium oxide. The signals at  $\delta_{\text{H}}$  11.16 and 4.41, which exchanged quickly, were assigned to one aromatic and one aliphatic hydroxy proton, whereas the signals at  $\delta_{\text{H}}$  10.46 and 10.31, which exchanged slowly, were assigned to two NH protons, this fact confirming the presence of two indole moieties.

Although in the 300 MHz  $^1\text{H}$  n.m.r. spectrum of (1) in  $\text{CDCl}_3$  not all the protons were resolved completely, all of them could be assigned as reported in Table 1. Several structural features were evident, such as the presence of four vicinal and two *ortho*-coupled ( $^3J$  8.0 Hz) aromatic protons belonging to the indole rings A' and A, respectively. Two of them could be rigorously attributed with the aid of the following n.O.e. experiments. (see Figures 1 and 2). Irradiation of the NH resonance at  $\delta_{\text{H}}$  8.27 resulted in enhancement of the signal at  $\delta_{\text{H}}$  7.46 (2.5%) which was, therefore, assigned to 12'-H, whereas irradiation of the NH resonance at  $\delta_{\text{H}}$  7.65 resulted in enhancement of the doublet at  $\delta_{\text{H}}$  6.68 (1.5%), permitting its assignment to 12-H. The latter



experiment was of cardinal importance in the determination of the substitution pattern of the indole ring A because it proved that this ring is disubstituted at C-9 and C-10. Furthermore, these experiments allowed a full distinction between some neighbouring protons from the two alkaloid portions. In fact irradiation of 1'-H enhanced also signals at  $\delta_{\text{H}}$  4.68 (0.5%), 2.31 (1.5%), and 1.75 (1%), whilst irradiation of 1-H enhanced the signal at  $\delta_{\text{H}}$  1.88 (1.5%); these signals were later assigned unambiguously to 3' $\beta$ -H, 14' $\alpha$ -H, 15' $\alpha$ -H, and 14 $\alpha$ -H respectively.

Extensive use of  $^{13}\text{C}$  n.m.r. spectroscopy was required for the elucidation and the connection of the two units. The

Table 1. <sup>1</sup>H N.m.r. data of a uncaramine (1) and gambirine (2) in CDCl<sub>3</sub>

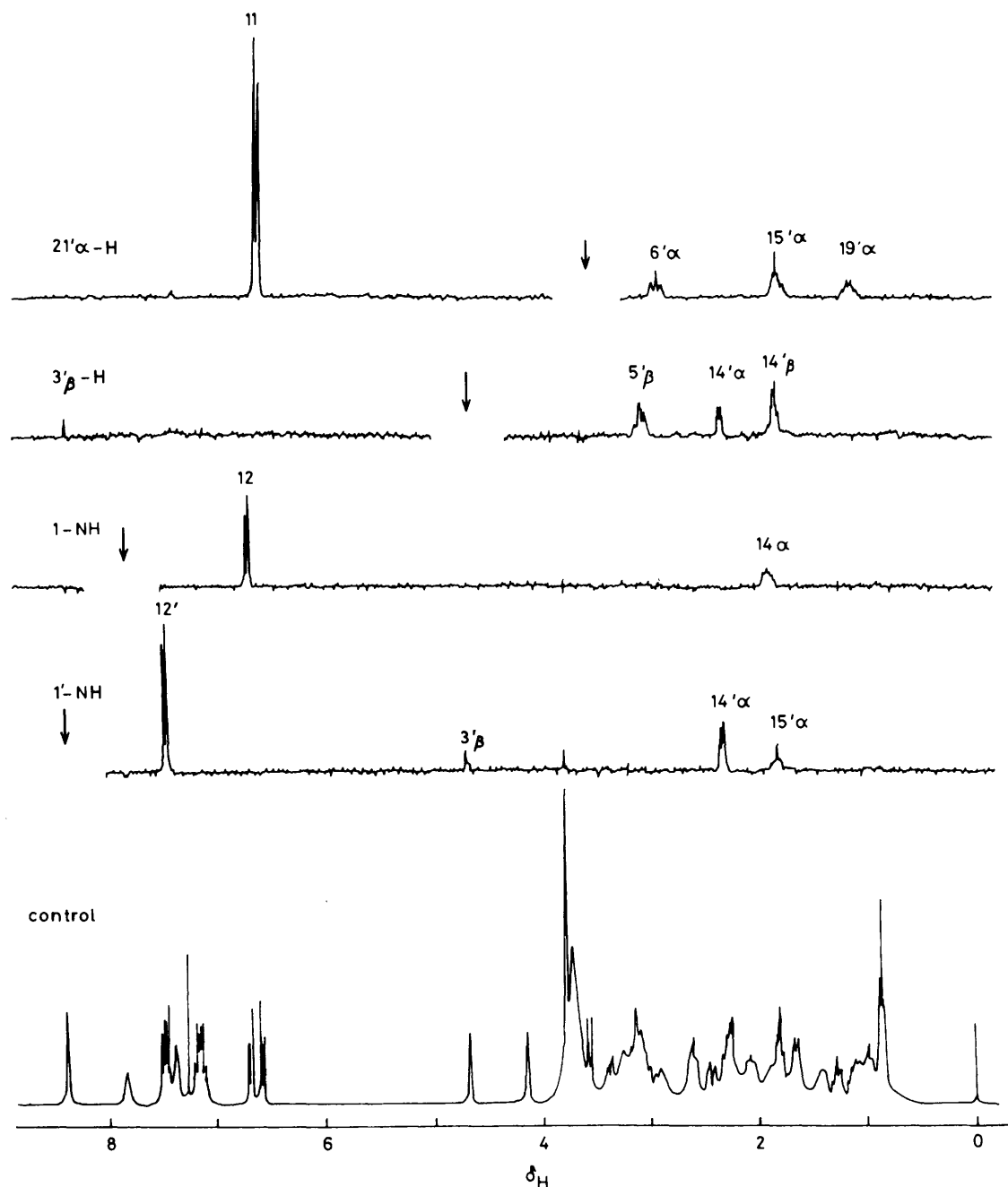
(1) <sup>a</sup>					(2)			
Proton	δ <sub>H</sub>	Proton	δ <sub>H</sub>	J(HH) Hz	Proton	δ <sub>H</sub>	J(HH)	Hz
1	7.65	1'	8.27	3'β,6'α	1	7.66	3α,6α	1.5
3α	3.27	3'β	4.68	3'β,6'β	3α	3.24	3α,6β	2.5
5α	2.63	5'α	3.39	3'β,14'α	5α	2.61	3α,14α	2.5
5β	ca. 3.1	5'β	3.08	3'β,14'β	5β	3.10	3α,14β	ca. 11.0
6α	ca. 3.1	6'α	2.91	5'α,5'β	6α	2.99	5α,5β	11.2
6β	3.25	6'β	2.45	5'α,6'α	6β	3.25	5α,6α	4.2
		9'	7.49	5'α,6'β	10	6.40	5α,6β	11.0
11	6.58	10'	7.13	5'β,6'α	11	6.91	5β,6α	1.5
12	6.68	11'	7.19	5'β,6'β	12	6.84	5β,6β	5.7
14α	1.88	12'	7.46	6'α,6'β	14α	1.90	6α,6β	15.0
14β	ca. 2.0	14'α	2.31	9',10'	14β	2.05	10,11	7.2
15α	2.63	14'β	1.83	9',11'	15α	2.63	10,12	1.0
17	7.36	15'α	1.75	10',11'	17	7.37	11,12	8.0
18	0.87	16'β	2.28	10',12'	18	0.86	14α,14β	12.5
19a	1.42	17'β	4.15	11',12'	19a	1.43	14α,15α	4.0
19b	1.02	18'α	1.67	14'α,14'β	19b	1.05	14β,15α	11.5
20β	2.30	18'β	1.29	14'α,15'α	20β	2.27	15α,20β	11.0
21α	2.09	19'α	1.11	14'β,15'α	21α	2.07	18,19a	7.2
21β	3.17	19'β	0.99	15'α,16'β	21β	3.15	18,19b	7.2
23	3.71 <sup>b</sup>	20'β	1.67	15'α,20'β	23	3.70 <sup>b</sup>	19a,19b	13.5
24	3.75 <sup>b</sup>	21'α	3.57	16'β,17'β	24	3.76 <sup>b</sup>	19a,20β	3.2
9-OH	11.16 <sup>c</sup>	23'	3.80	17'β,18'α	9-OH	9.03 <sup>c</sup>	19b,20β	8.7
		17'α-OH	4.41 <sup>c</sup>	17'β,18'β			20β,21α	11.0
				18'α,18'β			20β,21β	3.7
				18'α,19'α			21α,21β	11.0
				18'α,19'β				
				18'β,19'α				
				18'β,19'β				
				19'α,19'β				
				19'α,20'β				
				19'β,20'β				
				20'β,21'α				

<sup>a</sup> Owing to the complexity of the spectrum of (1), only  $J_{11,12} = 8.0$ ,  $J_{11,21'α} < 0.5$ ,  $J_{20β,21α} = 10.5$ ,  $J_{20β,21β} = 3.5$ , and  $J_{21α,21β} = 11.0$  Hz could be assigned for the gambirine unit. <sup>b</sup> Assignment within each column may be interchanged. <sup>c</sup> In (CD<sub>3</sub>)<sub>2</sub>SO. <sup>d</sup> Not assigned.

Table 2. <sup>13</sup>C N.m.r. data of uncaramine (1), dihydrocorynantheine (3), and pseudoyohimbine (4) in CDCl<sub>3</sub>

(1)			(3) <sup>a</sup>	(1)			(4) <sup>b</sup>
Carbon	δ <sub>C</sub> <sup>c</sup> /p.p.m.	<sup>1</sup> J(CH)/Hz	δ <sub>C</sub> /p.p.m.	Carbon	δ <sub>C</sub> <sup>c</sup> /p.p.m.	<sup>1</sup> J(CH)/Hz	δ <sub>C</sub> /p.p.m.
2	133.51 <sup>d</sup> S		135.2	2'	132.84 <sup>d</sup> S		132
3	60.35 D	<i>g</i>	60.2	3'	54.77 D	(140.5)	54.6
5	53.31 T	(137)	53.1	5'	47.91 T	(139)	51.0
6	23.70 T	(130)	21.9	6'	16.51 T	(129)	16.9
7	107.87 <sup>e</sup> S		107.5	7'	108.86 <sup>e</sup> S		108
8	117.49 S		127.4	8'	127.83 S		127
9	150.89 S		117.9	9'	118.06 D	(158.5)	117
10	113.19 S		120.9	10'	121.63 <sup>f</sup> D	(161)	121
11	124.33 D	(157.5)	119.0	11'	119.50 <sup>f</sup> D	(159)	119
12	101.56 D	(161)	110.8	12'	111.64 D	(160)	111
13	137.63 S		136.2	13'	136.50 S		135
14	33.93 T	(128)	33.8	14'	32.58 T	(128)	31.6
15	38.58 D	(128)	38.7	15'	31.10 D	(128)	32.5
16	112.20 S		111.7	16'	52.26 D	(128)	52.1
17	159.89 D	(179.5)	159.8	17'	67.06 D	(147.5)	67.0
18	11.23 Q	(125.5)	11.3	18'	31.91 T	(128)	31.4
19	24.47 T	(129)	24.4	19'	22.58 T	(128)	23.0
20	39.50 D	<i>g</i>	39.3	20'	43.22 D	(129)	40.2
21	60.96 T	<i>g</i>	61.3	21'	64.10 D	(137)	52.0
22	169.00 S		168.9	22'	174.75 S		174
23	51.21 Q	(146.5)	51.1	23'	51.81 Q	(147)	50.9
24	61.50 Q	(146.5)	61.3				

<sup>a</sup> From ref. 8. <sup>b</sup> From ref. 7. <sup>c</sup> Relative to SiMe<sub>4</sub>. Capital letters refer to the pattern resulting from one-bond (CH) couplings; S = singlet, D = doublet, T = triplet, and Q = quartet. <sup>d-f</sup> Assignments may be interchanged. <sup>g</sup> Not assigned.



**Figure 1.** Control spectrum and n.O.e. difference spectra for some irradiation in uncaramine (1). Irradiated protons are indicated at the left and marked by an arrow. The control spectrum is broadened by 1.0 Hz. Difference spectra are  $\times 64$  vertical display scale compared with the control

chemical shifts observed in the proton noise decoupled  $^{13}\text{C}$  n.m.r. spectrum and the multiplicities observed in the fully  $^1\text{H}$ -coupled  $^{13}\text{C}$  n.m.r. spectrum (see Table 2) indicated that compound (1) contains 4 methyl, 10 methylene, 16 methine, and 13 quaternary carbon atoms. Of the 20 resonances in the 100–175 p.p.m. region due to  $sp^2$ -hybridised carbon atoms, the 12 resonances belonging to the aromatic rings A and A' were readily assigned by selective irradiation of the appropriate hydrogen atoms. In particular, irradiation of the doublet signal at  $\delta_{\text{H}}$  3.57 (21' $\alpha$ -H) caused the doublet of doublets at  $\delta_{\text{C}}$  150.89 [ $^3J(\text{CH})$  8.5 and 5.5 Hz] to simplify to a doublet, the remaining coupling (8.5 Hz) being a three-bond coupling to 11-H. These findings, as corroborated by chemical shift considerations, allowed us to assign the decoupled signal to the hydroxy-

bearing carbon atom C-9 and established that C-10 and C-21' are involved in coupling between the two alkaloid halves of uncaramine. Accordingly, 21' $\alpha$ -H was also coupled to the proton-bearing carbon signal at  $\delta_{\text{C}}$  124.33 [ $^3J(\text{CH})$  4.5 Hz], assigned to C-11, and to the signal at  $\delta_{\text{C}}$  113.19 [ $^2J(\text{CH})$  3 Hz]. The latter, which was also coupled to 12-H [ $^3J(\text{CH})$  8 Hz] was assigned to C-10. The remaining signals at  $\delta_{\text{C}}$  137.63 and 117.49, presenting a three-bond coupling to 11-H [ $^3J(\text{CH})$  10 Hz] and respectively to 12-H [ $^3J(\text{CH})$  5 Hz], were attributed to C-13 and C-8. The chemical shifts of the remaining 8  $sp^2$ -hybridised carbon atoms were in agreement with the presence of two ester carbonyl carbon atoms, one trisubstituted double bond, and the 4 quaternary carbon atoms of ring B and B'. Having in our hands gambirine (2), whose structure was defined in 1967 using

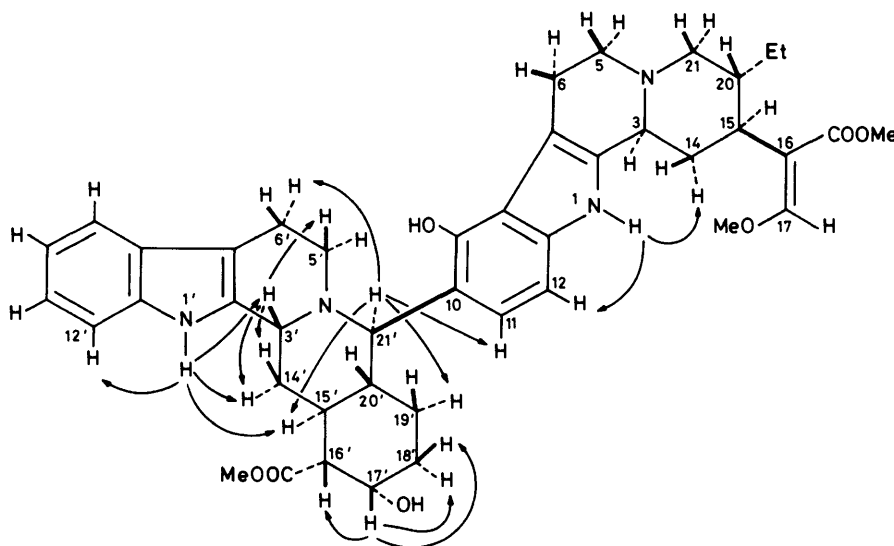


Figure 2. The n.O.e. connectivity pattern observed for uncaramine (1)

classical methods,<sup>1</sup> we have completely analysed its <sup>1</sup>H n.m.r. spectrum at 300 MHz with the aid of extensive homonuclear decoupling experiments.\* <sup>1</sup>H Chemical shifts and coupling constants which are collected in Table 1 confirmed that gambirine possesses a 'normal' configuration<sup>4</sup> (3 $\alpha$ , 15 $\alpha$ , and 20 $\beta$ ),<sup>†</sup> and established the preferred conformations of ring C and D as half-chair and chair, respectively.

Comparison of the <sup>1</sup>H chemical shifts of (1) and (2), and of the <sup>13</sup>C chemical shifts of (1) and dihydrocorynantheine (3),<sup>8</sup> apart from those arising from rings A and B resonances, revealed a close similarity between each pair of compounds. These findings, in conjunction with the above described n.m.r. study of rings A and B, identified the first unit of uncaramine as a 10-substituted gambirine. The structure elucidation of the second alkaloid unit, which was earlier demonstrated to be of yohimbine-type,<sup>2</sup> as well as the linking between the two alkaloid halves derived from the following evidence. The <sup>1</sup>H n.m.r. spectrum of this second portion of uncaramine exhibited, in addition to the previously analysed four vicinally coupled protons of ring A', to 1'-H, and to the aliphatic hydroxy proton, resonances due to 19 protons. <sup>1</sup>H-<sup>1</sup>H and <sup>13</sup>C-<sup>1</sup>H Low-power selective decoupling and n.O.e. experiments, coupled with chemical shift criteria and coupling constant values, permitted their full assignment (see Table 1). Thus the three-proton singlet at  $\delta_{\text{H}}$  3.80 (23'-H<sub>3</sub>) was readily attributed to the protons of a methyl ester and the broad singlet at  $\delta_{\text{H}}$  4.68, correlated to the resonance at  $\delta_{\text{C}}$  54.77, was assigned to the  $\beta$ -equatorially disposed 3'-H. Its irradiation allowed the assignment of 14'-H<sub>2</sub> (<sup>3</sup>J<sub>3',14' $\alpha$</sub>  3.0 and <sup>3</sup>J<sub>3',14' $\beta$</sub>  4.5 Hz) and the protons of ring C' because 3'-H presented homoallylic couplings to 6'-H<sub>2</sub> (<sup>5</sup>J<sub>3',6' $\alpha$</sub>  2.5 and <sup>5</sup>J<sub>3',6' $\beta$</sub>  1.5 Hz), which in turn were coupled to 5'-H<sub>2</sub>.

Furthermore irradiation of the signal at  $\delta_{\text{H}}$  2.28 (16'-H) caused the decoupling of the resonances due to the  $\alpha$ -disposed 15'-H ( $\delta_{\text{H}}$  1.75) and to the carbinyl 17'-H ( $\delta_{\text{H}}$  4.15), the values of the removed couplings (<sup>3</sup>J<sub>15',16' $\beta$</sub>  10.5 and <sup>3</sup>J<sub>16',17' $\beta$</sub>  2.5 Hz) being indicative of *trans*-diaxial and *gauche* relationships, respectively, between 15' $\alpha$ , 16' $\beta$ , and 16' $\beta$ , 17' $\beta$  protons. Spin-

decouplings also revealed that the  $\beta$ -equatorially disposed 17'-H was vicinally coupled to 17'-OH and 18'-H<sub>2</sub> [<sup>3</sup>J<sub>17' $\beta$ ,18' $\alpha$</sub>  3.0, <sup>3</sup>J<sub>17' $\beta$ ,18' $\beta$</sub>  2.1, and <sup>3</sup>J<sub>17' $\beta$ ,17' $\alpha$ -OH</sub> 4.5 Hz, this latter in (CD<sub>3</sub>)<sub>2</sub>SO] and that 18'-H<sub>2</sub> and 20'-H were bonded to carbons separated by the common C-19' methylene group. Additionally, the values of the coupling constants between 18' $\beta$ -H, 19' $\alpha$ -H, and 19' $\alpha$ -H, 20' $\beta$ -H (<sup>3</sup>J 12.0 and 11.0 Hz, respectively) indicated that each pair of protons are *trans*-diaxially disposed. The remaining proton at  $\delta_{\text{H}}$  3.57, which has been demonstrated to be located at the carbon linked to C-10 of the gambirine portion, was then assigned to 21'-H, the value of <sup>3</sup>J<sub>20' $\beta$ ,21' $\alpha$</sub>  (9.6 Hz) suggesting that the gambirine moiety is  $\beta$ -equatorially disposed at C-21'.

The magnitude of the couplings between 5'- and 6'-H<sub>2</sub>, namely <sup>3</sup>J<sub>5',6' $\beta$</sub>  < 2 and <sup>3</sup>J<sub>5',6' $\alpha$</sub>  12.0 Hz, and the n.O.e. between the *cis* pseudoaxial-disposed 3' $\beta$ -H and 5' $\beta$ -H (1.5%) (see Figure 1), indicated that ring C' is in a half-chair conformation whilst evidence for the *trans* C'/D' ring junction followed from the close agreement between the diagnostic chemical shift values for C-3' ( $\delta_{\text{C}}$  54.77), C-6' ( $\delta_{\text{C}}$  16.51), and C-14' ( $\delta_{\text{C}}$  32.58), all evaluated from low-power selective decouplings experiments, with the corresponding values reported for pseudoyohimbine (4).<sup>7,9</sup> On the other hand the magnitude of the coupling constants of the protons of rings D' and E' (Table 1) indicated that these two rings are both in a chair conformation and *trans*-fused. A n.O.e. experiment performed on the  $\alpha$ -axially positioned 21'-H provided conclusive proof of the above described stereochemistry. In fact its irradiation resulted in enhancement of the axially disposed 6' $\alpha$ -H (1.5%), 15' $\alpha$ -H (1.5%), and 19' $\alpha$ -H (1.5%), accompanied by the expected n.O.e. (4%) on 11-H of the gambirine unit (see Figures 1 and 2). It must therefore be concluded that uncaramine is a binary indole alkaloid formed by gambirine and pseudoyohimbine linked together through a C(10)-C(21') bond.

The downfield shift experienced by the 9-hydroxy proton ( $\Delta\delta$  2.13 p.p.m.) in the <sup>1</sup>H n.m.r. spectrum of (1) in (CD<sub>3</sub>)<sub>2</sub>SO in comparison with the corresponding resonance of gambirine as well as the small benzylic coupling constant (<0.5 Hz) between 11-H and 21'-H suggested the presence of an intramolecular hydrogen bonding between the phenolic hydroxy proton and a proton acceptor, and that 21'-H is positioned roughly in the plane of the indole ring A. These suppositions were substantiated by inspection of Dreiding models of compound (1) which revealed that both the above outlined requirements were fulfilled simultaneously since the formation of an intramolecular

\* The sample was warmed at 60 °C because at lower temperature the slow rotation of the groups bonded to C-15 and C-20 broadened some neighbouring signals, as well as in (1).

† It is obviously assumed that the absolute configuration of C-15 (and C-15') is  $\alpha$ .

C(9)OH...H<sub>b</sub>(4') hydrogen bond necessitates that 21'α-H be roughly coplanar with ring A. As a consequence the most striking conformational result is the observation of a strongly preferred orientation of the two halves of the molecule, the C(11)–C(10)–C(21')–H(21') dihedral angle being *ca.* 20°.

Uncaramine shows structural features of unusual interest, being the first example, to our knowledge, of a binary alkaloid derived from one unit of corynantheine and one of yohimbine-type.

### Experimental

Flash chromatography was performed with Merck silica gel (0.040–0.063 mm), and t.l.c. with Merck HF<sub>254</sub> silica gel. Mass spectra were taken on a VG-ZAB2 instrument at 70 eV. <sup>1</sup>H (300.13 MHz) and <sup>13</sup>C (75.47 MHz) N.m.r. spectra were recorded on a Bruker CXP-300 spectrometer. Chemical shifts are in p.p.m. (δ) from SiMe<sub>4</sub> as internal standard. N.O.e. difference spectra were obtained by subtracting alternatively right-off resonance-free induction decays (FIDS) from right-on resonance-induced FIDS. N.O.e. values reported in the text have only qualitative significance.

*Isolation and Purification of Uncaramine (1).*—The isolation and some physical constants of compound (1) have already been reported;<sup>2</sup> further purification was made by flash chromatography with CH<sub>2</sub>Cl<sub>2</sub>–MeOH (15:1), cream amorphous powder,

m.p. >300 °C (decomp.) (Found: C, 69.3; H, 6.7; N, 7.2%. C<sub>43</sub>H<sub>52</sub>N<sub>4</sub>O<sub>7</sub> requires C, 70.08; H, 7.11; N, 7.60%); [α]<sub>589</sub> +20°, and [α]<sub>436</sub> +45.6° (*c* 0.2 in CHCl<sub>3</sub>); *m/z* 736 (*M*<sup>+</sup>), 533, 384, 383, 368, 353, 284, 184, 154, and 138 (Found: *M*<sup>+</sup>, 736.383 ± 0.001. C<sub>43</sub>H<sub>52</sub>N<sub>4</sub>O<sub>7</sub> requires 736.387; 383.1967 ± 0.002. C<sub>22</sub>H<sub>27</sub>N<sub>2</sub>O<sub>4</sub> requires 383.1970; 353.1807 ± 0.01. C<sub>21</sub>H<sub>25</sub>N<sub>2</sub>O<sub>3</sub> requires 353.1865).

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Received 20th March 1986; Paper 6/544