[Chem. Pharm. Bull. 25(8)2067—2071(1977)]

UDC 547.945.02 : 581.192

Studies on Plants containing Indole Alkaloids. VI.[1] Minor Bases of *Uncaria rhynchophylla* MIQ.

NORIO AIMI, ETSUJI YAMANAKA, NOBUO SHINMA, MORIO FUJIU, JUNKO KURITA, SHIN-ICHIRO SAKAI, and JOJU HAGINIWA

Faculty of Pharmaceutical Sciences, Chiba University[2]

(Received December 24, 1976)

Two minor alkaloids were newly isolated from *Uncaria rhynchophylla* MIQ., and their structures elucidated as geissoschizine methyl ether (a new compound) and akuammigine, respectively.

The Cotton effect due to the chirality of the E ring of heteroyohimbinoid alkaloids was studied by using some model compounds. The conformation of akuammigine was also studied.

Keywords——*Uncaria rhynchophylla* MIQ.; Rubiaceae; structure determination; indole alkaloids; heteroyohimbinoids; stereochemistry; conformational analysis; CD; geissoschizine methyl ether; akuammigine

Previously we reported our investigations on the alkaloidal constituents of *Uncaria rhynchophylla* MIQ.[3] In that paper the main bases rhynchophylline (**1**) and isorhynchophylline (**2**) were shown to be accompanied by the almost equal amounts of the corresponding dehydro analogues, corynoxeine (**3**) and isocorynoxeine (**4**). At the same time four indolic alkaloids namely hirsutine (**5**), hirsuteine (**6**), corynantheine (**8**) and dihydrocorynantheine (**7**), were isolated and fully characterized. In connection with the importance of this plant, which has been used as a crude drug for a long time in traditional oriental medicine, further investigations of the minor constituents have been made.

Purification of the basic extract by repeated column chromatography afforded a new base, base I (**9**), as colorless prisms, mp 190—192°, $C_{22}H_{26}N_2O_3$, (0.01%). Its ultraviolet (UV) spectrum was characteristic for an indolic alkaloid possessing a β-alkoxy acrylic ester function. On the nuclear magnetic resonance (NMR) spectrum, signals due to carbomethoxyl and enolic methoxyl functions were observed at δ 3.72(3H,s) and 3.80(3H,s). The presence of an ethylidene group was revealed by a pair of signals at δ 1.54(3H,d,J=7Hz, $=\mathrm{C}\langle{}^{\mathrm{H}}_{\underline{\mathrm{Me}}}$) and 5.40(1H,q,$J$=7Hz, $=\mathrm{C}\langle{}^{\underline{\mathrm{H}}}_{\mathrm{Me}}$). Bohlmann bands were observed between 2850 and 2730 cm^{-1} in the infrared (IR) spectrum of **9**, evidence for the α configuration of $C_{(3)}$-H. This conclusion was further supported by its circular dichroism (CD) spectrum, which showed a positive Cotton effect at 270—300 nm.[4]

On reduction with Adams' catalyst in acetic acid, **9** gave two known compounds, dihydrocorynantheine (**7**) and corynantheidine (**10**), which were identified with the respective authentic specimens by mixed melting point measurement and comparison of their IR and CD spectra. All the evidence stated above suggested that base I was the hitherto unknown methyl ether

---

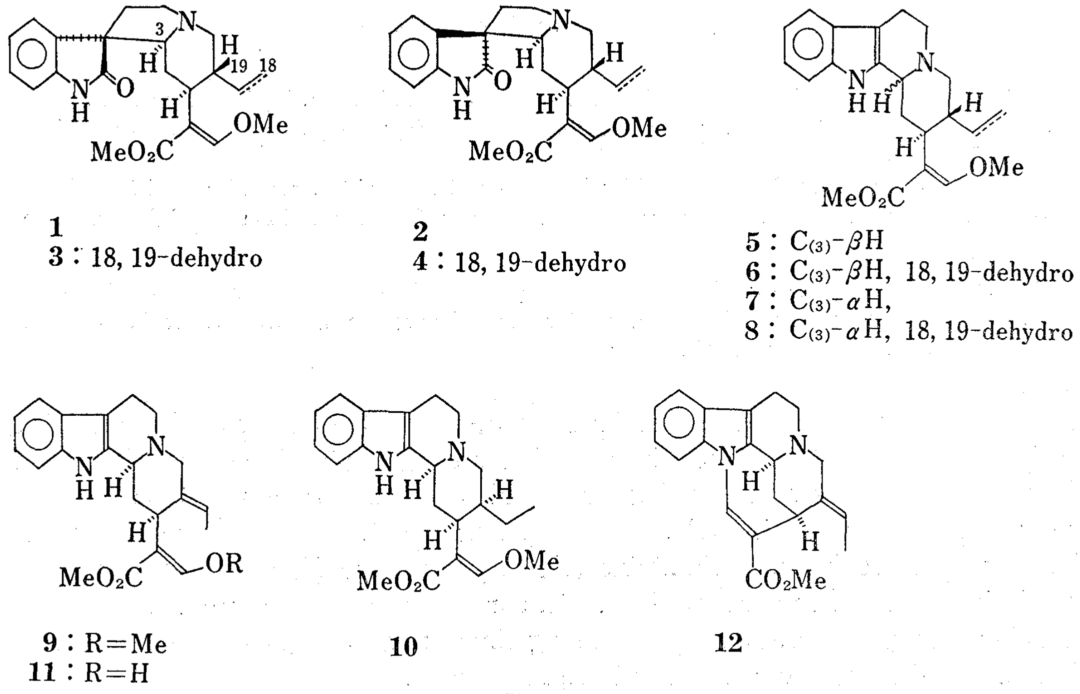
1) Part V: S. Sakai, N. Aimi, K. Kato, H. Ido, K. Masuda, Y. Watanabe, and J. Haginiwa, *Yakugaku Zasshi*, **95**, 1152 (1975).

2) Location: *1-33, Yayoi-cho, Chiba.*

3) J. Haginiwa, S. Sakai, N. Aimi, E. Yamanaka, and N. Shinma, *Yakugaku Zasshi*, **93**, 448 (1973).

4) W. Klyne, R.J. Swan, N.J. Dastoor, A.A. Gorman, and H. Schmid, *Helv. Chim. Acta*, **50**, 115 (1967).

(**9**) of geissoschizine.[5] Mass spectral fragmentation of **9** is also in good accord with this structure. To determine the geometry of the $C_{(19)}=C_{(20)}$ double bond, cleavage of the enolic ether linkage of **9** was made by use of acetone–hydrogen chloride. The spectral properties of the resulting product (**11**), mp 190—191°, showed good agreement with the reported values of geissoschizine whose geometry of $C_{(19)}=C_{(20)}$ double bond is known to be E.[6] A small amount of apogeissoschizine(**12**)[5] was also formed in this reaction. As geissoschizine(**11**) is known to be one of the most important intermediates in indole alkaloid biosynthesis, the isolation of its methyl ether from a natural source is of great interest. Furthermore, this compound (**9**) has been found to be an important starting material in our chemical conversion work, details of which have been published in the separated papers.[7,8]



1
3 : 18, 19-dehydro

2
4 : 18, 19-dehydro

5 : $C_{(3)}$-βH
6 : $C_{(3)}$-βH, 18, 19-dehydro
7 : $C_{(3)}$-αH,
8 : $C_{(3)}$-αH, 18, 19-dehydro

9 : R=Me
11 : R=H

10

12

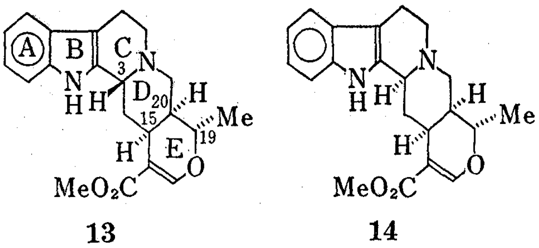
Chart 1

Another base, base II (**13**), was obtained as an amorphous powder (0.001%). When heated in acetic acid, **13** was equilibrated with its $C_{(3)}$ epimer, and the latter was proved to be tetrahydroalstonine (**14**). This fact strongly suggested that base II was akuammigine (**13**). Correctness of the assumption was unambiguously proved by the comparison of its spectral properties with those reported on akuammigine (**13**)[9,10] and direct comparison with an authentic specimen which had been obtained in our conversion work using pteropodine (**18**) and isopteropodine (**19**) as the starting materials.[11]

Akuammigine (**13**) belongs to *epiallo* type ($C_{(3)}$-βH,$C_{(20)}$-αH) of the heteroyohimbinoid alkaloids and its conformation is controversical since it can take two conformations whose energy difference is small. As a sample of akuammigine was available a study on its conformation by CD spectrometry was undertaken.

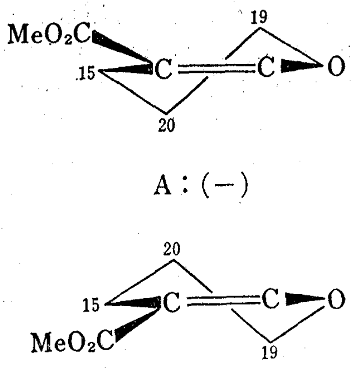
---

5) H. Rapoport, R.J. Windgassen, N.A. Hughes, and T.P. Onak, *J. Am. Chem. Soc.*, **82**, 4404 (1960).
6) K. Yamada, K. Aoki, T. Kato, D. Uemura, and E.E. van Tamelen, *Chem. Commun.*, **908**, (1974).
7) S. Sakai, E. Yamanaka, and L.J. Dolby, *Heterocycles*, **4**, 981 (1976).
8) S. Sakai and N. Shinma, *Heterocycles*, **4**, 985 (1976).
9) "Physical Data of Indole and Dihydroindole Alkaloids," ed. by N. Neuss, Eli Lilly Co., Indianapolis, 1974.
10) For the physical data and discussions on its structure see: E. Wenkert, B. Wickberg, and C.L. Leicht, *J. Am. Chem. Soc.*, **83**, 5036 (1961); M. Shamma and J.M. Richey, *J. Am. Chem. Soc.*, **85**, 2507 (1963); L. Merlini, R. Mondelli, G. Nassini, and M. Hesse, *Tetrahedron*, **26**, 2259 (1970).
11) N. Aimi, E. Yamanaka, J. Endo, S. Sakai, and J. Haginiwa, *Tetrahedron*, **29**, 2015 (1973).



13 **14**

Chart 2



A : (−)

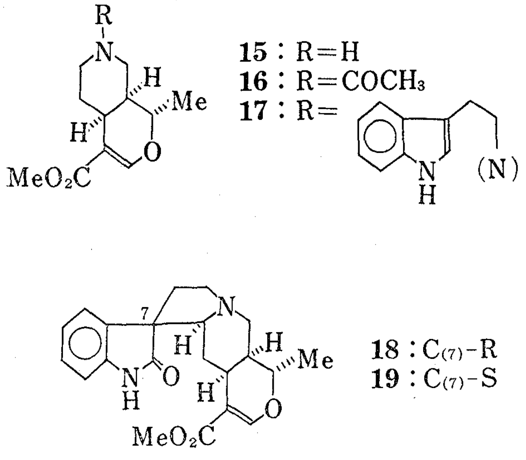
B : (+)

Chart 3

In 1966 Klyne, *et al.*[12] reported a study on optical rotatory dispersion (ORD) of heteroyohimbinoid alkaloids. In that paper they ascribed the Cotton effect at 235—255 nm to the chirality of the E rings. The perspective views of the two possible conformations of the E ring are shown as below, and they concluded that the compound having A type conformation showed minus Cotton effect and B type plus.

The chirality of E ring is related to the relative stereochemical arrangement among the four asymmetric centres, *i. e.* C$_{(3)}$, C$_{(15)}$ (always S-configuration), C$_{(19)}$ and C$_{(20)}$. Therefore the sign of the Cotton effect of this chromophore gives an important information regarding to the stereostructures of this group of compounds.

In the treatment of Klyne, *et al.* all of their materials have two dissymmetric chromophores, *i.e.* the indole ring and the α,β-unsaturated ester group in the same molecule, and only the ORD spectra were measured. As a consequence the curves were rather complicated and as they themselves admit, there remained some uncertainty regarding the sign and amplitude of the Cotton effect due to the isolated β-alkoxy acrylic ester moiety of the molecules. In order to clarify this point, CD spectra were measured on some model compounds (**15**—**17**) which retained β-alkoxy acrylic ester as the only dissymmetric chromophore. All of these model compounds were derived from the known oxindole alkaloids, pteropodine (**18**) and isopteropodine (**19**), through a route established in this laboratory.[13] The wavelengths and $\Delta\varepsilon$ values of their CD maxima are shown in Table I.



15 : R=H
16 : R=COCH$_3$
17 : R=
18 : C$_{(7)}$-R
19 : C$_{(7)}$-S

Chart 4

Table I. CD Maxima

Compounds	λ (nm)	$\Delta\varepsilon$
15	230	−3.6
16	230	−5.6
17	243	−7.0

---

[12] N. Finch, W.I. Taylor, T.R. Emerson, W. Klyne, and R.J. Swan, *Tetrahedron*, **22**, 1327 (1966).

[13] See a review article; S. Sakai, *Heterocycles*, **4**, 131 (1976). A full report of this part is in preparation.

Each of compounds (**15**—**17**) can take two conformations (A and B), and the conformation A with an equatorial C-methyl group can be regarded as the prefered one. Uskoković synthesized (**15**) and its N-benzoate in racemic forms and ascribed them A conformation from the NMR evidences.[14)]

When the Klyne rule was applied to each of these possible conformations the observed negative CD signs (Table I) favor the A conformation, which is also compatible with Uskoković's conclusion. Thus the present study strongly supported validity of Klyne's rule.

Then CD spectrum of akuammigine was measured. A weak positive Cotton effect was observed at 248 nm($\Delta\varepsilon$ +0.9) between two negative ones at 271 nm ($\Delta\varepsilon$ −2.6) and 231 nm ($\Delta\varepsilon$ −11.4). In the paper of Klyne, the only member of the *epiallo* series on which ORD measurement was made was reserpilline (**20**) and a weak positive Cotton effect was also observed. This fact is consistent with the previous conclusion that akuammigine exists in the equilibrium between two conformations, form A (*cis* quinolizidine) and form B (*trans* quinolizidine), in which the latter is more dominant.[15)]

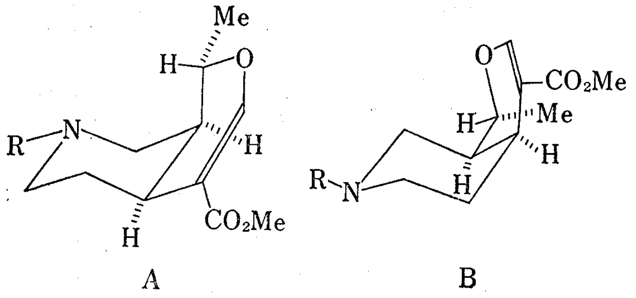
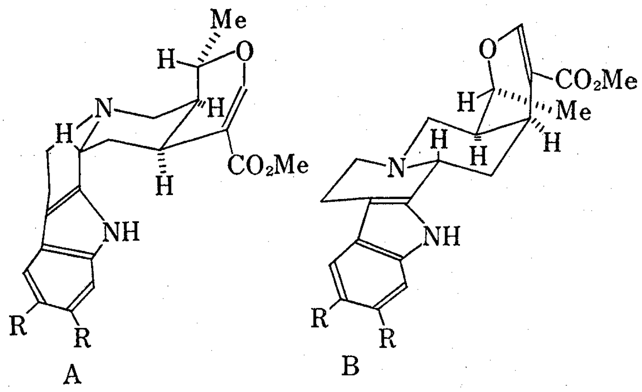


Chart 5



13 : R=H
20 : R=OMe

Chart 6

Experimental

All melting points are uncorrected. IR spectra were measured with a Model 215 spectrophotometer (Hitachi Co.) and UV spectra with a Model EPS-3T spectrophotometer (Hitachi Co.). A JNM MH-100 NMR instrument (Japan Electron Optics Co.) was used for measurement of NMR spectra using $CDCl_3$ as the solvent. Mass spectra (MS) were taken with a Model RMU-6E mass spectrometer (Hitachi Co.). Measurement of CD spectra was made by using a Model J-20 spectropolarimeter (Japan Electroscopic Co.) on methanol solutions, concentrations of which were around 1 mg/10 ml.

Extraction and Separation of Alkaloids——Powdered whole plant of *Uncaria rhynchophylla*, which had been mixed with $Ca(OH)_2$ and steamed for about 3 hr, was extracted with hot benzene. The concentrated benzene extract was shaken with dilute sulfuric acid (2%) and the acid layer was basified with NH_4OH. Extraction with $CHCl_3$ afforded the crude base as heavy syrup. As already reported, purification of the crude base using column chromatography on Al_2O_3 and SiO_2 afforded following bases; corynantheine, dihydrocorynantheine, hirsutine, hirsuteine, corynoxeine, rhynchophylline, isocorynoxeine and isorhynchophylline. The main fraction, from which most of hirsutine and hirsuteine had been removed, was submitted to further purification using silicic acid column chromatography and two more minor bases were obtained as described below.

Base I (Geissoschizine Methyl Ether) (9)——From the fraction eluted with $CHCl_3$–$(CH_3)_2CO$ (7:1) and (6:1) colorless prisms, mp 190—192° (acetone–hexane), were obtained, 0.01% (from the dry plants). This base was an unknown compound and was proved to be geissoschizine methyl ether (**9**) from the following physicochemical data and conversion to known compounds. Geissoschizine methyl ether, mp 190—192°.

---

14) J. Gutzwiller, G. Pizzolato, and M. Uskoković, *J. Am. Chem. Soc.*, **93**, 5907 (1971).

15) *a*) G.A. Morrison, "Vortschritte der Chemie Organischer Naturstoffe," L. Zechmeister Ed., Springer Verlag, Wien, XXV, 1967, p. 269, and references cited therein; *b*) E. Wenkert, C-J. Chang, H.P.S. Chawla, D.W. Cochran, E.W. Hagaman, J.C. King, and K. Orito, *J. Am. Chem. Soc.*, **98**, 3645 (1976).

$[\alpha]_D^{27}$ +100° (c=1.0, MeOH). UV λ_{max}^{EtOH} nm (log ε): 227 (4.66), 251 (sh, 4.12), 284 (sh, 3.93), 291 (3.84). MS *m/e*: 366 (M^+, 100%), 351 (43%), 337 (90%), 325 (34%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3475 (NH), 2850—2730 (Bohlmann band), 1700, 1640 (conj. ester). NMR δ 1.54 (3H, d, $C_{(19)}$–Me), 3.72 (3H, s, OMe), 3.80 (3H, s, OMe), 5.40 (1H, q, $C_{(19)}$–H). CD (c=0.01) λ_{max} nm ($\Delta\varepsilon$): 294 (+2.6), 270 (+4.8), 236 (+16.1), 222 (−17.2). *Anal.* Calcd. for $C_{22}H_{26}N_2O_3$: C, 72.10; H, 7.15; N, 7.65. Found: C, 72.06; H, 7.20; N, 7.51.

Demethylation of Base I (9)——Dry hydrogen chloride was passed into an ice-cold solution of 500 mg of **9** in 50 ml of dry acetone to saturation. The resulting solution was kept standing at −13°. A solution of 424 mg of **9** in 40 ml of dry acetone was treated in the same way as above. After 24 hr both solutions were poured into 300 ml of ice-water and extraction was made four times with 20 ml of $CHCl_3$ to remove HCl salt of the starting **9**. The aqueous layer was made basic with conc. NH_4OH and the solution was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried with anhydrous sodium sulfate and the solvent was removed *in vacuo*. The residue (900 mg) thus obtained was chromatographed over 30 g of neutral Al_2O_3 (Woelm, activity III). Elution with benzene gave 155 mg of minor product (**12**) (yield, 20%). Elution with benzene and benzene–$CHCl_3$ (7: 1) gave 98 mg of the starting material. Elution with MeOH and MeOH–AcOH (6%) gave 361 mg of the major product (**11**) (yield, 33%), which on crystallization from EtOH gave 191 mg of pure (**11**). The mother liquor from the above crystallization was submitted to preparative TLC and 14 mg of pure (**11**) was obtained after crystallization from EtOH. The total yield of **11** was 205 mg (yield, 26%).

The physical and spectral properties of the minor and major products, **12** and **11**, were as follows, and these data were in good accord with the reported data on apogeissoschizine (12)$^{5)}$ and geissoschizine (11) respectively.

Minor product (apogeissoschizine) (12). Picrate, mp 186—187° (from EtOH). *Anal.* (Picrate), Calcd. for $C_{27}H_{25}N_5O_9$; C, 57.54; H, 4.47; N, 12.43. Found, C, 57.07; H, 4.60; N, 12.13. MS *m/e*: 334 (M^+, 100%), 319 (M^+−Me, 14%), 292 (94%), 278 (58%), 266 (48%). UV λ_{max}^{EtOH} nm: 224.5, 277 and 325. $\lambda_{max}^{EtOH+10\%\ HCl}$ nm: 219, 272.5 and 323. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no NH; 1690, 1625 (conj. ester). NMR δ: 8.30 (1H, s, $C_{(17)}$–H), 5.36 (1H, q, J=7 Hz, $C_{(19)}$–H), 4.32 (1H, d, $C_{(15)}$–H), 4.04 (1H, br. s, $C_{(3)}$–H), 3.76 (3H, s, CO_2Me), 1.76 (3H, d, J=7 Hz, $C_{(19)}$–Me).

Major product (geissoschizine) (11): mp 190—191° (dec.) (from EtOH). MS *m/e*: 352 (M^+, 100%), 323 (M^+−CHO, 50%), 251 (99%), 250 (23%), 184 (19%), 170 (35%), 169 (59%), 156 (34%). UV λ_{max}^{EtOH} nm (log ε): 224 (4.61), 268 (4.20), 290 (3.89). $\lambda_{max}^{EtOH+dil.\ NaOH}$ nm: 227.5, 278 (with increased intensity). IR ν_{max}^{KBr} cm^{-1}: 3345 (NH), 1650 (C=O). NMR δ: 1.80 (3H, d, J=6 Hz, $C_{(19)}$–Me), 3.70 (3H, s, CO_2Me), 4.50 (1H, d, J=12 Hz, $C_{(3)}$–H or $C_{(15)}$–H), 5.40 (1H, q, J=6 Hz, $C_{(19)}$–H), 7.84 (1H, s, $C_{(17)}$–H), 8.12 (1H, s, NH).

Catalytic Reduction of Base I (9)——87 mg of **9** in acetic acid (8 ml) was hydrogenated with Adams' catalyst at room temperature for 4 hr. The catalyst was removed by filtration and the filtrate was basified with NH_4OH. Extraction with $CHCl_3$ afforded a residue which was submitted to preparative TLC on SiO_2 plates using benzene–$CHCl_3$ (8: 1) as the developer. Dihydrocorynantheine, mp 85—90° (10 mg) and corynantheidine, mp 74—75° (15 mg) were obtained and they were identified with the respective authentic samples by mixed mp measurement and comparison of the IR and CD spectra.

Base II (Akuammigine) (13)——From the column described in the head of this experimental base II was eluted with $CHCl_3$–$(CH_3)_2CO$ (7: 1) to give an amorphous powder (0.001% from the dry material). The physical properties are as follows; $[\alpha]_D^{27}$ −41° (c=1.0, EtOH). UV λ_{max}^{EtOH} nm: 226, 283.5, 291. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (NH), 1690, 1620 (conj. ester). MS *m/e*: 352 (M^+, 100%), 351 (60%), 337 (45%), 251 (35%), 223 (47%), 169 (45%), 156 (70%). NMR δ: 1.30 (3H, d, J=6 Hz, $C_{(19)}$–Me), 3.70 (3H, s, OMe), 4.38 (1H, quintet, J=6 Hz, $C_{(19)}$–H), 7.51 (1H, s, $C_{(17)}$–H). CD (c=0.01), λ_{max} nm ($\Delta\varepsilon$): 271 (−2.6), 248 (+0.9), 231 (−11.4). These data were in good accord with those reported for akuammigine (13), and the identity was proved by direct comparison of IR spectra and TLC with an authentic specimen.