UDC 547.759

53. Toshikazu Nozoye: Studies on Uncaria Alkaloid. XVIII. Structure of Uncarine. (13).[1] Structure of Uncarine-A and Uncarine-B.

(*ITSUU Laboratory**)

Uncarine-A and -B, the alkaloid of *Uncaria Kawakamii* HAYATA, have the same composition and are represented by the following empirical formula.[2]

$$C_{21}H_{24\sim26}O_4N_2{}^{2a)} = C_{18}H_{21\sim23}\begin{cases}\equiv N \\ -CO-NH- \\ -COOCH_3 \\ -O-\end{cases}\begin{cases}\text{One C-CH}_3\text{ group} \\ \text{No N-CH}_3\text{ group} \\ \text{One active hydrogen (Zerewitinoff)}\end{cases}$$

Uncarine-A : Amorphous. Hydrochloride, m.p. 220°(decomp.), $[\alpha]_D^{23}$ +113.1°
Uncarine-B : Prisms (from acetone), m.p. 216~217°.
Hydrochloride, m.p. 227~228°(decomp.), $[\alpha]_D^{23}$ +93.6°.

These two alkaloids are easily isomerizable into the other,[2a,3] give the same decomposition product, and their ultraviolet and infrared spectra are so similar that they can be considered as stereoisomers with identical structure.

It has been found that the parent structure of uncarine contains an oxindole and piperidine rings since dehydrogenation of the alkaloid over palladium[3,4] affords β-collidine (I) and 3,4-diethylpyridine (II),[5] and because oxindole-3-spiro-1′-cyclopropane[6]

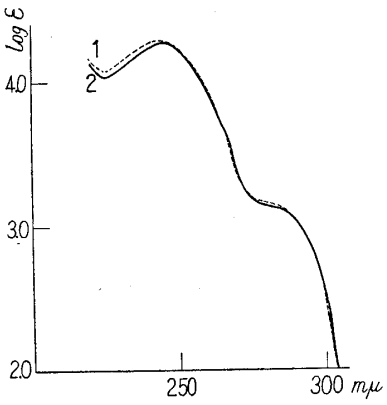


Fig. 1. Ultraviolet Spectra (in MeOH)
Curve 1 : Uncarine-A
Curve 2 : Uncarine-B

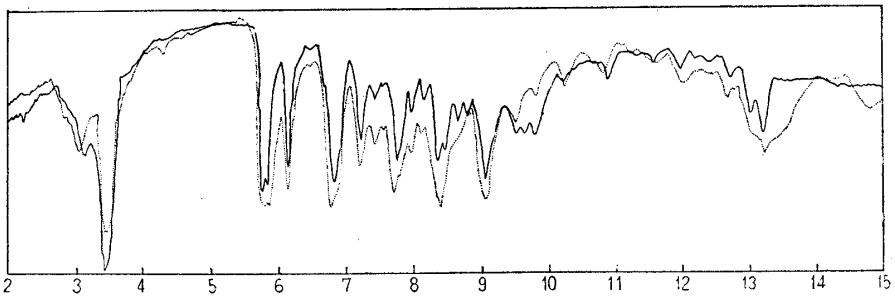


Fig. 2. Infrared Spectra (in Nujol)
····· Uncarine-A —— Uncarine-B

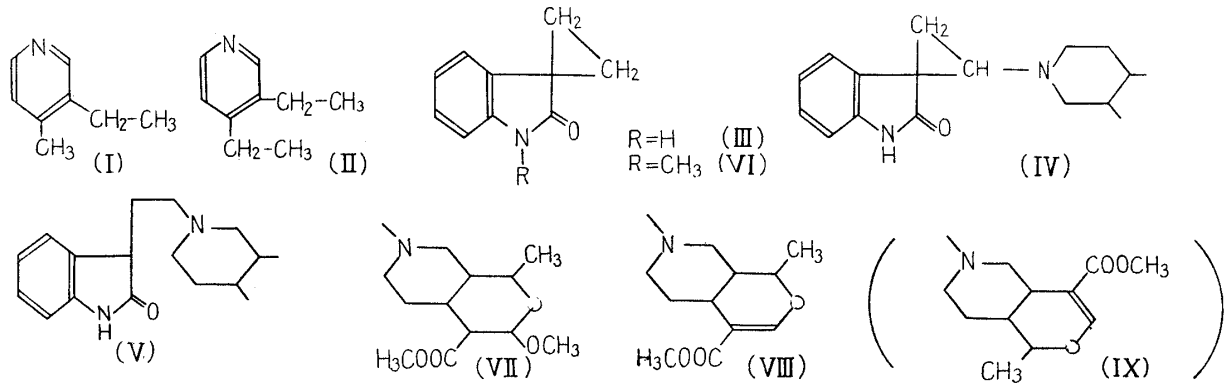
* Konno-cho, Shibuya-ku, Tokyo (野副利一).

1) Part (12). H. Kondo, T. Nozoye: Ann. Rept. ITSUU Lab. (Tokyo), **7**, 44(1956).
2) *a*) H. Kondo, T. Ikeda: Yakugaku Zasshi, **61**, 416(1941); *b*) H. Kando, T. Nozoye, M. Tobita: Ann. Rept. ITSUU Lab. (Tokyo), **5**, 84(1954).
3) H. Kondo, T. Nozoye: *Ibid.*, **1**, 71(1950).
4) Tetsutaro Ikeda: Yakugaku Zasshi, **61**, 460(1941).
5) Determined by mixed fusion with the picrate recently supplied by Prof. P. Karrer.
6) *a*) H. Kondo, T. Nozoye, M. Tobita: Ann. Rept. ITSUU Lab. (Tokyo), **5**, 80(1954). *b*) H. Kondo, T. Nozoye, H. Tsukamoto: *Ibid.*, **6**, 53(1955).

(III) is formed by palladium-dehydrogenation under a reduced pressure[3] or by low-pressure sublimation of the methiodide.[3]

As to the bonding of these two rings, possibility of (IV) or (V) was considered, but the structure of (IV) was completely denied since the low-pressure pyrolysis of the methiodide of 1-methyl-3-(2-diethylaminoethyl)oxindole, the model compound, afforded 1-methyloxindole-3-spiro-1'-cyclopropane (VI)[1] and the infrared absorption of a cyclopropane ring conjugated to benzene ring, appearing at 945~960 cm^{-1},[7] does not show in the spectrum of uncarine. The structure of (V) will give two active hydrogens and this will be against the value for 1 in uncarine. Therefore, only a partial structure was indicated in the preceding paper.[1]

The four carbon atoms of the side chain in the piperidine ring, methoxycarbonyl group, and ether oxygen were indicated as (VIII) or (IX),[2b] considering the suggestion of a $-C(COOCH_3)=CH-O-$ bond in the ultraviolet and infrared absorption spectra,[2b] and the suggetsion of a characteristics of a dihydropyran ring by the formation of methoxydihydrouncarine-B[8] by adding methanol in the presence of acid,[2b] and the presence of one $C-CH_3$ group.[9] By analogy with other alkaloids possessing a similar structure such as alstonine,[10]



serpentine,[11] aricine,[12] melionine-A,[13] and corynantheine,[14] and from considerations on the biogenesis, the structure (VIII) is believed to be correct.

In order to experimentally prove this point, uncarine was reduced with lithium aluminum hydride and attempt was made to obtain the evidence by dehydrogenation of the reduction product. The product obtained by the reduction of uncarine-A with lithium aluminum hydride corresponded to its octahydro compound and its ultraviolet absorption indicated it to be an indoline derivative (Fig. 3). The N-methyl derivative of this octahydro compound was obtained in a crystalline state, which took CH_3OH when recrystallized from methanol.

Julian[15] has examined the reduction of oxindole derivatives with lithium aluminum hydride, and it is now known that indole is formed when there is hydrogen in 3-position while indoline is formed if both 3-positions are substituted with alkyl groups. This has accidentally provided the proof that there is no hydrogen in the 3-position of the

7) T. Nozoye : *Ibid.*, **8**, 48(1957).
8) This compound had been designated as methyluncarinol-B (H. Kondo, Tetsutaro Ikeda : Yakugaku Zasshi, **61**, 424(1941)) which is changed as above.
9) Tetsutaro Ikeda : Yakugaku Zasshi, **62**, 21(1942).
10) F. E. Bader : Helv. Chim. Acta, **36**, 215(1953).
11) F. E. Bader, H. Schwarz : *Ibid.*, **35**, 1594(1952).
12) R. Goutarel, M. M. Janot, V. Prelog : *Ibid.*, **37**, 1805(1954).
13) E. Schlittler : *Ibid.*, **35**, 29(1952).
14) P. Karrer, R. Schweyzer, A. Flamm : *Ibid.*, **35**, 851(1952).
15) P. L. Julian, H. L. Printy : J. Am. Chem. Soc., **71**, 3206(1949).

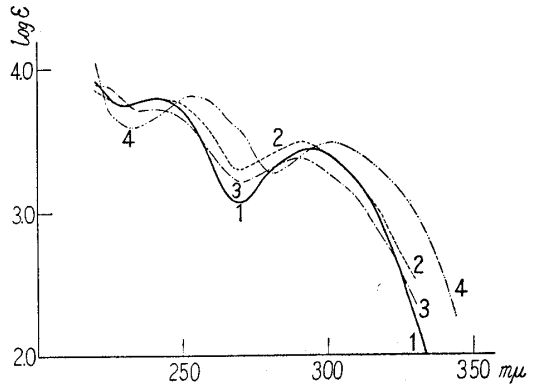


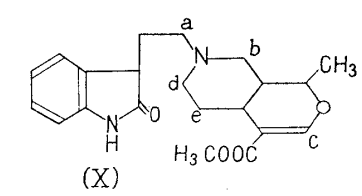
Fig. 3. Ultraviolet Spectra (in MeOH)

1. Octahydrouncarine-A
2. Octahydrouncarine-B
3. Demethoxycarbonyluncarine-B
4. Reduction product of (III)

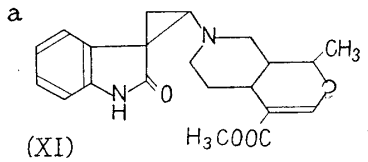
indole ring in the uncarine molecule. For further confirmation, lithium aluminum hydride reduction of uncarine-B, demethoxycarbonyluncarine-B, and (III) was carried out and all the products obtained indicated the presence of an indoline in their ultraviolet absorption spectra (Fig. 3).

For further chemical evidence, uncarine-A and -B were each reacted with methyl iodide in the presence of sodium methoxide. Uncarine-A gave N-methyluncarine-A (methiodide was produced on reaction over a long period but was not obtained in crystalline form) and uncarine-B gave N-methyluncarine-B methiodide, both in crystalline form and their analytical values were in good agreement with these. If a hydrogen is present in 3-position, the reaction should have produced 1,3-dimethyl derivative and this experiment has also proved the spiro-type bonding at 3-position.

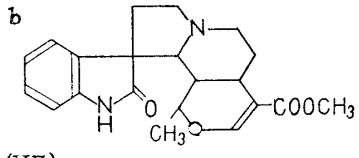
The positions capable of bonding with the carbon at 3-position was examined by atom model and five positions, a, b, c, d, and e, in (X) seemed possible.



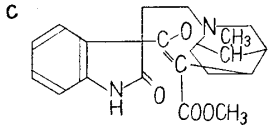
(X)



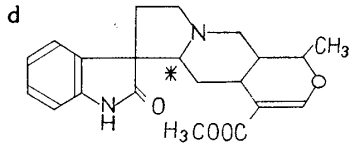
(XI)



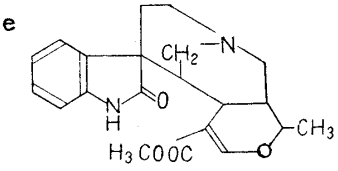
(XII)



(XIII)



(XIV)



(XV)

In the case of a, the structure would be indicated by (XI) but this requires a cyclopropane ring conjugated with the benzene ring and this had been denied by infrared spectrum.[7]

In the case of b, the structure would be (XII) but this has an unavoidable defect for the following facts. In spite of the fact that uncarine contains an oxindole ring, which has been proved to be present in lactam ring from its infrared absorption,[2b] it is extremely sparingly soluble in alkali hydroxide, especially uncarine-B, which is almost insoluble. On the other hand, demethoxycarbonyluncarine-B (XVI) is soluble in alkali hydroxide, like oxindole derivatives in general, and further shows peculiar behavior. For example, treatment of the methiodide (XVII) of (XVI) with alkali hydroxide causes fixation of the lactim form to take the structure (XVIII) which is proved by the absence of a carbonyl band and new absorptions, assumed to originate in =C-O- (1570 cm^{-1}), and one for C=N (1625 cm^{-1}) appear in its infrared absorption spectrum (Fig. 4) (When the

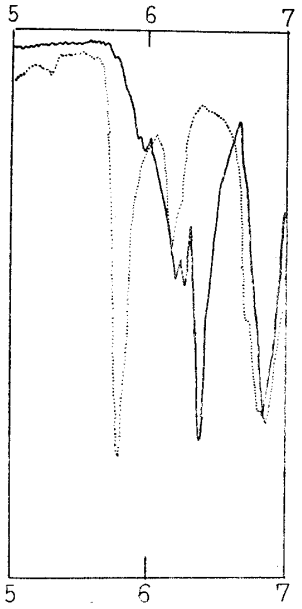
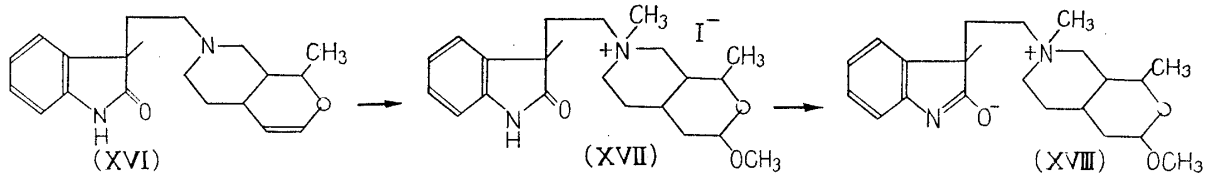


Fig. 4. Infrared Spectra (in Nujol)

—— (XVIII)
····· (XVII)

methiodide is produced in methanol solution, addition of CH_3OH to the dihydropyran ring occurs at the same time). This peculiar phenomenon is not observed in either uncarine-A or -B. It must, therefore, be considered that the methoxycarbonyl group



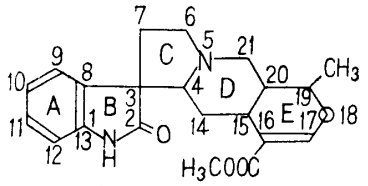
is situated close to the lactam in oxindole, with the carbonyl of the methoxycarbonyl group and NH in oxindole in hydrogen bonding, and prevented from forming a lactim. Therefore, it would be impossible to consider a structure like (XII) in which the methoxycarbonyl group is situated far from and on the opposite side of the oxindole ring.

(XIII) would represent c-type bonding but this structure is very rigid and there are no bonding that could produce epimers like uncarine-A and -B that this structure cannot be adopted.

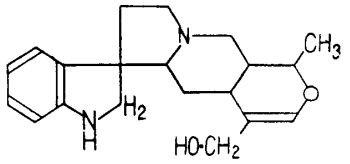
In the case of d, the structure would be indicated by (XIV) and epimers may be formed by the configuration of the hydrogen at the position marked with an asterisk, while e would be represented by (XV) in which the presence of epimers can be considered by the inversion of the bridged methylene adjacent to nitrogen. This leaves the choice of (XIV) or (XV) for the structure of uncarine.

A very helpful experimental result was provided for this selection by the measurement of pKa for uncarine-A and -B. The value of pKa was 4.2 for uncarine-A and 5.5 for uncarine-B, indicating a marked difference between the two. It had already been observed that there is a difference in the basicity of the nitrogen between the two,[3] since uncarine-B easily formed a methiodide while a prolonged heating of uncarine-A was necessary for formation of its methiodide. This was brought out more clearly by the pKa values. Moreover, the values of pKa of various derivatives of uncarine-B, such as 5.4 for methoxydihydrouncarine-B and 5.9 for demethoxycarbonyluncarine-B, are not greatly different from that of uncarine-B. It seemed more likely, therefore, that the low pKa value of uncarine-A is because the oxygen of the carbonyl in the oxindole is in close proximity of nitrogen and exerts a great effect on its basicity. This explana-

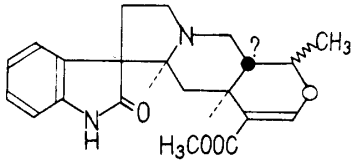
tion became more certain when it was found that the pKa of octahydrouncarine-A, (XX), obtained by the reduction of uncarine-A with lithium aluminum hydride, is 6.2 and vastly different from that of uncarine-A, and that pKa of octahydrouncarine-B (XX) is 6.5, there being little difference between the two octahydro compounds. From these facts, it became necessary that the carbonyl in oxindole and N_b are in very close proximity to each other and the structure that would satisfy all these conditions would leave only (XIV). Therefore, uncarine-A and -B would be represented by the structure (XIX).



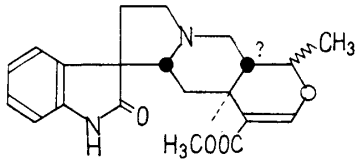
(XIX)



(XX)

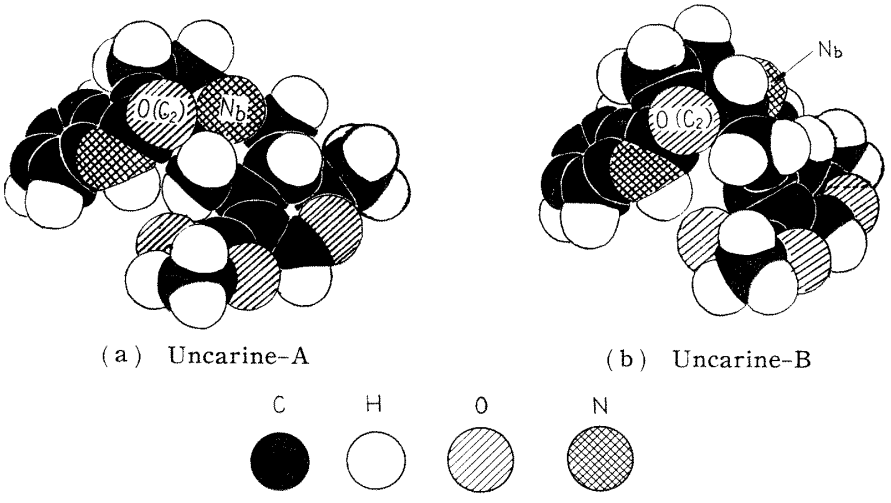


(XXI)



(XXII)

In (XIX), when the absolute configuration of hydrogen in 4-position is α-form, the oxygen and N_b in 5-position would be in closest location and this should be given to the structure of uncarine-A (Fig. 5a). It follows that uncarine-B would be its 4-epimer, i.e. the structure in which the hydrogen in 4-position takes the β-form (Fig. 5b). In



(a) Uncarine-A (b) Uncarine-B

Fig. 5. Atom Models

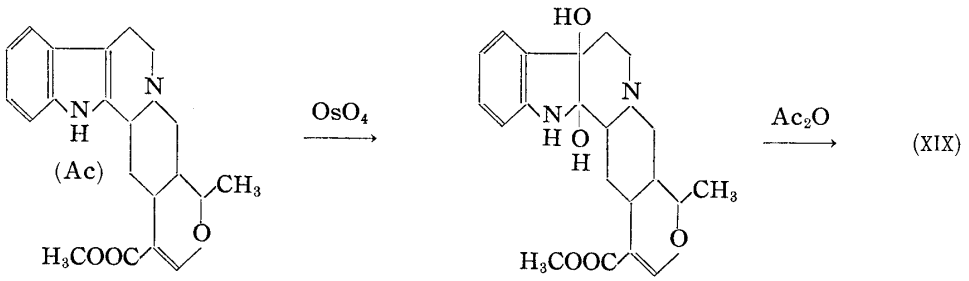
this case, if the hydrogen at 15-position in uncarine-B is taken as the β-form, then the D-ring would be in boat form and that would be impossible from the facile isomerization of uncarine-A into -B. Therefore, this can be considered to take the α-form. There is still no decisive evidence for the conformation of the hydrogen at 20-position but if the 15—20 ring juncture is considered to take the *allo* form, the tosyl ester of octahydrouncarine (XX), like reserpinol,[16] should contain $-CH_2-O$-tosyl group

16) C.F. Hebner, E. Wenkert : J. Am. Chem. Soc., **77**, 4180(1955); E.E. Van Tamelen, P.D. Hance: *Ibid.*, **77**, 4692(1955); P.A. Diassi, C.M. Dylion, F.L. Weisenborn, O. Wintersteiner : *Ibid.*, **77**, 4687(1955).

in such close proximity of N_b that $-CH_2-$ would be bonded to N_b and the tosyl group would form the tosylate of N_b. Actually, however, such a phenomenon has not been observed and the hydrogen in 20-position is more likely to be in β-form, with 15—20 juncture present as a normal type.

As a result of the foregoing considerations, the structure (XXI) is given for uncarine-A and (XXII) for uncarine-B.

The conversion of tetrahydrocarbazole to oxindole-3-spiro-1′-cyclopentane, first studied by Plant and Robinson,[17] has recently been extended by Van Tamelen[18] to tetrahydro-β-carboline, and its application to alkaloids of this system may establish the conformation of 15—20 juncture together with establishment of the structure. It is very likely that uncarine would be obtained by the conversion of tetrahydroalstonine or tetrahydroserpentine (XXIII), the former, considered to have the 15—20 juncture in normal type, seems more possible. Experimental results from these alkaloids are awaited with great expectations.



(XXIII)

The biogenesis of uncarine could well be explained by the strychnine-type condensation between 2-hydroxytryptamine and 3,4-dihydroxyphenylacetaldehyde, formation of a piperidine ring by formaldehyde, and further Woodward-cleavage of the dihydroxyphenyl ring, as in alstonine or serpentine.

The author expresses his deep gratitude to Prof. Dr. H. Kondo for kind encouragement and guidance throughout the coure of this work and to Prof. E. Ochiai for valuable advices. He is deeply indebted to Prof. P. Karrer of the University of Zürich for kind donation of a valuable sample. The author's thanks are due to Mr. S. Tanaka, Engineering Faculty, University of Tokyo, for infrared spectral measurements, to Mr. Keizo Watanabe of this Laboratory for ultraviolet spectral measurements and elemental analyses, and to Miss K. Kobayashi for the measurement of pK. Expenses for the present work were defrayed by the Grant in Aid for Miscellaneous Scientific Researches from the Ministry of Education which is gratefully acknowledged.

Experimental

Octahydrouncarine-A (XX)—A solution of 1 g. of uncarine-A dissolved in 10 cc. of ether was added into a solution of 0.5 g. of $LiAlH_4$ dissolved in 20 cc. of ether with stirring, during 1 hr. The mixture was then refluxed for 5 hrs., allowed to stand over night, and excess $LiAlH_4$ was decomposed by the addition of AcOEt. The organic solvent was distilled off, the residue was dissolved in dil. H_2SO_4 solution, basified with Na_2CO_3, and extracted with ether. After drying, the ether was evaporated and the residue was sublimed at a reduced pressure. The fraction subliming at 190~200°(bath temp.) at 15 mm. Hg was collected. Yield, 0.7 g. *Anal.* Calcd. for $C_{20}H_{26}O_2N_2$ (XX) : C, 73.57; H, 8.04; N, 8.57. Found : C, 73.63; H, 8.27; N, 8.15.

Methylation of Octahydrouncarine-A (N-Methyl-methoxydihydro-octahydrouncarine-A)—A mixture of 1 g. of octahydrouncarine-A, 3 cc. MeOH, and 1.5 cc. MeI was warmed on a water bath for 30 mins., allowed to stand over night, and the solvent was distilled off. The residue was basified with $NaHCO_3$, extracted with ether, and ether was evaporated after drying over anhyd. Na_2SO_4. The residue was recrystallized from MeOH to crystals melting at 109° with decomposition. Yield, 0.15 g. *Anal.* Calcd. for $C_{21}H_{29}O_2N_2(OCH_3)$: C, 70.98; H, 8.61; N, 7.53; OCH_3, 8.32. Found : C, 71.62; H, 9.05; N, 7.19; OCH_3, 8.14.

17) S. G. P. Plant, R. Robinson : Nature, **165**, 36(1950).
18) E. E. Van Tamelen : Chem. & Ind. (London), **1956**, 1145.

Application of Methyl Iodide to Uncarine-A in the Presence of Sodium Methoxide (N-Methyluncarine-A)—To MeOH solution of NaOMe, prepared from 0.13 g. of metallic Na and 10 cc. MeOH, 1 g. of uncarine-A and 1.5 g. MeI were added and the mixture was warmed on a water bath for 30 mins. After leaving over night, the solvent was distilled off, the residue was extracted with ether, and ether was evaporated from the extract after drying over anhyd. Na_2SO_4. Recrystallization of the ether residue from MeOH gave 0.1 g. of crystals, m.p. 160°. *Anal.* Calcd. for $C_{21}H_{23}O_3N_2(OCH_3)$: C, 69.09; H, 6.8; N, 7.35; OCH_3, 8.12. Calcd. for $C_{22}H_{27}O_3N_2(OCH_3)$: C, 69.32; H, 7.59; N, 7.03; OCH_3, 7.79. Found: C, 68.79; H, 6.8; N, 7.0; OCH_3, 8.68.

Application of Methyl Iodide to Uncarine-B in the Presence of Sodium Methoxide (N-Methyluncarine-B Methiodide)—To a solution of 1 g. of uncarine-B dissolved in MeOH solution of NaOMe, prepared from 0.13 g. of metallic Na and 10 cc. MeOH, 1.5 g. of MeI was added and the mixture was warmed on a water bath for 1 hr. The solvent was distilled off, the residue was extracted with ether, and ether was evaporated after drying over anhyd. Na_2SO_4. There was no residue. The ether-insoluble substance was recrystallized from MeOH and 1.2 g. of crystals of m.p. 228°(decomp.) was obtained. *Anal.* Calcd. for $C_{21}H_{23}O_3N_2 \cdot CH_3I(OCH_3)$: C, 52.65; H, 5.58; N, 5.35; OCH_3, 5.92. Calcd. for $C_{22}H_{27}O_3N_2 \cdot CH_3I(OCH_3)$: C, 53.31; H, 6.17; N, 5.18; OCH_3, 5.74. Found: C, 52.32; H, 5.85; N, 5.20; OCH_3, 6.27.

Summary

A spiro-type ring was found to be attached to the 3-position of the oxindole ring in uncarine-A and -B from the fact that lithium aluminum hydride reduction of these compounds afforded indoline derivatives and that the application of methyl iodide in the presence of sodium methoxide afforded 1-methyluncarine-A and 1-methyluncarine-B methiodide. Examination was made as to which carbon in the uncarine molecule this spiro bonding existed and the possibilities of the formulae (XIV) and (XV) were considered. However, measuremnt of pK revealed that N_b and the carbonyl in the oxindole were in very close proximity in the uncarine-A molecule. Relationship between uncarine-A and -B would be well explainable by assuming 4-epimeric structures and conformational formulae (XXI) for uncarine-A and (XXII) for uncarine-B were forwarded.

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54. Toshikazu Nozoye: Studies on Uncaria Alkaloid. XIX.[1] On Mitraphylline.

(*ITSUU Laboratory**)

When extracting uncarine from *Uncaria Kawakamii* HAYATA, a minute quantity of an alkaloid, sparingly soluble in acetone, is obtained. A fair amount of this alkaloid was accumulated and its repeated recrystallization from methanol afforded a substance of single unity as needle crystals, m.p. 266°, $[\alpha]_D^{23.5}$ +3.8°, and composition of $C_{21}H_{24\sim26}O_4N_2$ was suggested from its analytical values.

This composition and physical constants were in good agreement with those of mitraphylline, obtained first from *Mitragyna macrophylla*[2] and then from *Mit. stipulosa*.[3] Mixed fusion of this substance with the sample, m.p. 265°, kindly sent by Dr, Raymond-Hamet to Dr. Tetsutaro Ikeda, then of this Laboratory, and comparison of ultra-

* Konno-cho, Shibuya-ku, Tokyo (野副利一).

1) Part XVIII: This Bulletin, **6**, 300(1958).
2) L. Michiels: J. pharm. Belg., **13**, 719(1931) (C. A., **26**, 3070(1932)).
3) Raymond-Hamet, L. Millat: Bull. sci. pharmcol., **42**, 602(1935) (C. A., **30**, 1379(1936)).