THE STRUCTURE AND STEREOISOMERISM OF THREE MITRAGYNA ALKALOIDS[1]

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ABSTRACT

Isorhyncophylline, the isomer into which rhyncophylline is convertible, has been found to occur in nature. Both bases are interconvertible. Isorhyncophylline on hydrolysis with dilute hydrochloric acid is converted to an aldehyde reducible to isorhyncophyllol. When the aldehyde is reduced in the Wolff–Kishner reaction, it is also isomerized and the product is isorhyncophyllane. This reduction product is oxidized by mercuric acetate to a neutral dilactam which still contains the oxindole carbonyl and further contains a new lactam carbonyl present in a six-membered ring. Reduction of the dilactam with lithium aluminum hydride gave a product having the spectroscopic properties of an indole. This confirms the assumption previously made that in rhyncophylline, ring C is five-membered. The isomerization of rhyncophylline, mitraphylline, and formosanine is described. Formosanine has been shown to be identical with uncarine-B and thus uncarine-A is the iso base derivable from formosanine.

It has been reported first by Kondo, Fukuda, and Tomita (1) that rhyncophylline in acetic acid isomerizes to isorhyncophylline. It has now been possible to isolate isorhyncophylline from *Adina rubrostipulata* K. Schuman. It separated from the mother liquors that had already yielded rhyncophylline and mitraphylline. It was isolated as the perchlorate from which the crystalline base was liberated. Its ultraviolet spectrum was identical with that of rhyncophylline, and the infrared spectra of the two bases were very similar so that the isomerization did not seem to involve the chromophoric groups. When either rhyncophylline or its isomer was refluxed in pyridine it was converted to a mixture consisting of 30% rhyncophylline – 70% isorhyncophylline.

Whereas on catalytic hydrogenation rhyncophylline is converted to hexahydrorhyncophylline, in which the enol-ether double bond has survived, the similar hydrogenation of isorhyncophylline converts it to octahydroisorhyncophylline. The ultraviolet spectrum of this product showed only end absorption while the infrared spectrum contained absorption bands due to an unconjugated ester group (1743 cm^{-1}) and an oxindole carbonyl (1700 cm^{-1}) although it contained no absorption attributable to a benzene ring or an enol ether.

Hydrolysis of isorhyncophylline with dilute hydrochloric acid yielded a product having the properties of an aldehyde (a precipitate with Brady's reagent and restoration of color to Schiff's reagent), but it was not possible to induce the base or a salt to crystallize. The base appeared to polymerize on standing for a short time.

Reduction of the crude product, however, with sodium borohydride gave a base isomeric with rhyncophyllol (2), i.e., isorhyncophyllol ($C_{19}H_{26}O_2N_2$), the picrate of which crystallized. The regenerated base was a glass and its infrared spectrum in chloroform showed bands at 3610 cm^{-1} (hydroxyl), 3425 cm^{-1} (imino group), 1717 cm^{-1} (oxindole carbonyl), and 1628 cm^{-1} (benzene ring).

The amorphous aldehydic product of the hydrolysis of isorhyncophylline (i.e. isorhyncophyllal) when subjected to the Wolff–Kishner reaction suffered reduction of its aldehyde group. The product (73% yield) was identical with rhyncophyllane ($C_{19}H_{26}ON_2$) obtained previously (2) from rhyncophylline by the same reactions. In view of the fact that the thermal rearrangement of rhyncophylline and of rhyncophyllol gives mixtures in which

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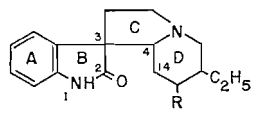
the isobase always predominates, it is more likely that this common product is isorhyncophyllane. Consequently, rhyncophyllane would have been more correctly named isorhyncophyllane, and we propose to adopt the latter designation.

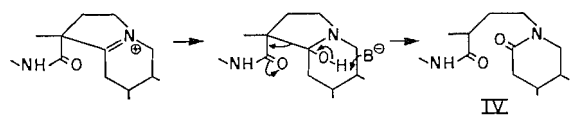
The structure of rhyncophylline represented by formula I has been suggested previously (2). From the foregoing results isorhyncophylline must also be represented by formula I



I, R = $CH_3OOC—C{=}CH.OCH_3$
II, R = $—CH_2.CH_2OH$
III, R = $.CH_2CH_3$

although the stereochemistry of the bases is different while isorhyncophyllol must be II and isorhyncophyllane III. Methylation with sodium methoxide converted isorhyncophyllane to N-methylisorhyncophyllane, which was crystallized as its picrate, m.p. 182–184°.

The final evidence needed to complete the proof that rhyncophylline (2) and its stereoisomer isorhyncophylline have structure I was the point of linkage of C-3 to ring D, and this has now been provided. Isorhyncophyllane reacted quickly with 1 mole of mercuric acetate giving rise to a neutral dilactam which in the infrared showed bands at 1715 cm^{-1} (oxindole carbonyl) and at 1625 cm^{-1} (six-membered lactam). This dilactam could not be crystallized, nor could it be purified completely because of partial decomposition when it was dissolved in a hot solvent. Lithium aluminum hydride in refluxing dioxane reduced the dilactam to a sensitive base which could not be purified without decomposition, but had an infrared spectrum showing no carbonyl absorption and an ultraviolet spectrum similar to an indole spectrum. The indication of indole formation on reduction, although not rigorous, does show, if taken in conjunction with the formation of a lactam in the reaction with mercuric acetate, the location of the new carbonyl. Thus the evidence is consistent with structure IV for the dilactam which must have been produced via the immonium ion and



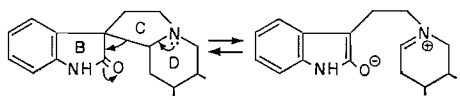
IV

carbinolamine as shown. It further establishes the five-membered nature of ring C.

Perhaps the most intriguing feature of rhyncophylline chemistry is the isomerization to isorhyncophylline. That the enol–ether system was not involved was shown by the fact that rhyncophyllol isomerizes in identical manner. Nozoye (3, 4) has suggested that the isomerism involves the configuration at C-4. A more plausible explanation of this phenomenon seemed to us to be the following thermal (possibly also acid-catalyzed) fission-recoupling process,[3] which involves the two asymmetric centers C-3 and C-4.

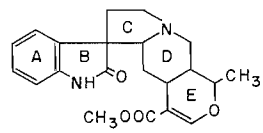
The pair of structures which represent rhyncophylline and its isomer must satisfy the requirements that the equilibrium between the two lies 70% on the side of the iso series,

[3]*This mechanism was recently independently suggested by Wenkert, Udelhofen, and Bhattacharyya (5).*



that rhyncophylline and derivatives are inert to mercuric acetate while the iso series react rapidly, and that the rhyncophylline series are stronger bases than the iso series. A rigorous assignment of stereochemical structures is not yet possible.[4]

An equilibrium mixture of 20% mitraphylline and 80% isomitraphylline is formed when either base is refluxed in pyridine overnight. It has not been possible to obtain isomitraphylline crystalline, but its picrate was a well-defined compound and the analytical figures obtained for this salt corresponded to $C_{21}H_{24}O_4N_2$ for the base. The ultraviolet spectrum of isomitraphylline was almost identical with that of mitraphylline (6) and the infrared spectra of the two bases showed no significant shift in the frequencies of the bands corresponding to the functional groups. Isomitraphylline when oxidized with mercuric acetate gave an amorphous product which on hydrogenation over Adams' catalyst produced a mixture of isomitraphylline and mitraphylline. The isomerism parallels that of rhyncophylline. Mitraphylline has been shown (6) to possess structure V, which must also represent isomitraphylline.



V

The alkaloid formosanine was isolated from *Orouparia formosana* Mats by Raymond-Hamet (7). It has the formula $C_{21}H_{24}O_4N_2$ and contains one methoxyl group. It has been suggested by Loudon (8) and by Raymond-Hamet (9) that uncarine-B isolated by Kondo (10) from *Uncaria kawakamii* Hayata was identical with formosanine because their melting points, rotations, and ultraviolet spectra were almost identical. We have now been able to make a direct comparison of formosanine[5] with a sample of uncarine-B kindly supplied by Professor H. Kondo. The melting point of the mixture of the bases showed no depression and their X-ray powder diagrams were identical while their infrared absorption spectra were virtually identical. This confirms the claim of Raymond-Hamet (9) that formosanine and uncarine-B are identical.

The pair of alkaloids uncarine-A–uncarine-B (formosanine) have been assigned structure V (3), which is also that of mitraphylline (6). When boiled with pyridine, formosanine is equilibrated to a mixture of about 20% formosanine and 80% isoformosanine. Formosanine is isomeric with mitraphylline, but not identical with it. Whereas mitraphylline was hydrogenated catalytically to hexahydromitraphylline in which the

[4]*An analysis of the stereochemistry is possible, and leads to a conclusion respecting the structures of the two isomers. This speculation, however, is based on rather arbitrary assumptions which will require further experimental confirmation.*

[5]*Dr. Raymond-Hamet kindly gave us a generous sample of formosanine and we acknowledge our indebtedness and gratitude.*

isolated double bond was still present, catalytic hydrogenation converted formosanine to octahydroformosanine.

The three alkaloids rhyncophylline, mitraphylline, and formosanine have a very low solubility in ether, and crystallize readily, while the bases of the iso series are very soluble in ether and either crystallize with difficulty or not at all. The bases equilibrate under the same conditions and in the mixtures produced, the iso base always predominates. Furthermore, the normal series are stronger bases than the iso series. It is therefore most probable that the isomerization in each case is of the same type.

It has been shown (3) that formosanine (uncarine-B) possesses the same structure V as mitraphylline, and also that uncarine-A (isoformosanine) is different from isomitraphylline or its isomer. Hence mitraphylline and formosanine must differ by an isomerism other than that differentiating mitraphylline from isomitraphylline.

The infrared spectra were measured on a Perkin–Elmer double-beam spectrophotometer model 21. Unless otherwise mentioned the spectra were taken in Nujol mulls, and the absorption bands are reported in wave numbers sometimes followed by a numeral in parentheses indicating the percentage absorption.

EXPERIMENTAL

Isolation of Isorhyncophylline

Both rhyncophylline and mitraphylline have already been reported as occurring in *Adina rubrostipulata* K. Schuman. The mother liquors from which the two bases had separated still contained basic material which was precipitated with picric acid. A sample of the crude picrate was suspended in methanol and shaken with Amberlite IRA 400 (basic form) and the mixture poured on a column of Amberlite IRA 400. Elution with methanol gave a solution of bases which was evaporated to dryness under diminished pressure. The basic residue was dissolved in the minimum volume of methanol and the solution made just acid to Congo red with perchloric acid. About half the solvent was evaporated and water added to incipient turbidity. After cooling and scratching a salt crystallized which was recrystallized from methanol from which it separated as colorless needles, m.p. 158–160°. It did not depress the melting point of an authentic sample of isorhyncophylline perchlorate prepared from rhyncophylline by isomerization. Found: C, 52.84; H, 6.19; N, 5.45. Calc. for $C_{22}H_{28}O_4N_2.HClO_4.H_2O$: C, 52.53; H, 6.21; N, 5.57%.

To liberate the base, the perchlorate was suspended in aqueous ammonia and the mixture extracted repeatedly with ether. The extract was washed with water, evaporated to dryness, and the residue dissolved in ether–hexane from which it crystallized as colorless fine needles, m.p. 150°. The infrared spectrum of the free base, 3420 cm^{-1} (HN), 1730 cm^{-1} (ester carbonyl), 1705 cm^{-1} (oxindole carbonyl), 1645 cm^{-1} (enol-ether), 1625 and 755 cm^{-1} (benzene ring), was identical with that of isorhyncophylline, and so were the ultraviolet spectra, $[\alpha]_D^{28}$ +5.9 (*c*, 3.95 in ethanol). Found: C, 68.89; H, 7.40; N, 7.21. Calc. for $C_{22}H_{28}O_4N_2$: C, 68.72; H, 7.34; N, 7.29%.

Octahydroisorhyncophylline

Isorhyncophylline (100 mg) was hydrogenated in glacial acetic acid (10 ml) over Adams' catalyst (100 mg) at 27° and a pressure of 755 mm of mercury. The absorption of hydrogen amounted to 26.7 ml and the volume calculated for four double bonds was 25.8 ml. After removal of the catalyst and solvent the residue was alkalized with sodium carbonate and the mixture extracted with ether. The extract was dried, the solvent

evaporated, and the residue crystallized in ether from which it separated as colorless prisms, m.p. 169–172°, $[\alpha]_D^{24}$ +46.9° (*c*, 0.49 in ethanol). Found: C, 67.33; H, 9.14. Calc. for $C_{22}H_{36}O_4N_2$: C, 67.31; H, 9.24%. Infrared spectrum: 1745, 1700, and 1110 cm^{-1}. Ultraviolet spectrum: end absorption.

Isomerization of Isorhyncophylline

Isorhyncophylline (280 mg) was refluxed with acetic anhydride (6 ml) for 3 hours. The solvent was removed under diminished pressure, the residue dissolved in benzene and chromatographed on alumina. Elution with benzene–ether gave a fraction (200 mg) which failed to crystallize, but formed a perchlorate which, after crystallization from methanol–ether, melted at 219–220° identical with isorhyncophylline perchlorate by mixed melting point and comparison of infrared spectra. Further elution with chloroform yielded rhyncophylline, m.p. 209–211° identical with an authentic specimen.

Equilibration of Rhyncophylline and Isorhyncophylline

Rhyncophylline (342 mg) was refluxed overnight in pyridine (7 ml). The solvent was evaporated off, the residue dissolved in ether, and the solution seeded with rhyncophylline. The crystalline base (96 mg) identified with rhyncophylline by melting point, mixed melting point, and infrared spectrum was filtered and the filtrate made just acid with perchloric acid. The crystalline salt (330 mg) was identical with isorhyncophylline perchlorate. Hence the equilibrium mixture contains approximately 30% rhyncophylline and 70% isorhyncophylline.

Isorhyncophyllol

It has been shown earlier (2) that rhyncophylline is converted to rhyncophyllal by the action of dilute hydrochloric acid. In an attempt to prepare isorhyncophyllal, isorhyncophylline (1.65 g) was refluxed similarly with 8% hydrochloric acid (200 ml) for 3.5 hours. The cooled solution was neutralized with sodium bicarbonate and extracted with ether. The extract was dried over sodium sulphate and evaporated to dryness. The residual gum was soluble in ether, acetone, benzene, methanol, and ethanol, but much less in hexane. After a short time it became only partially soluble in ether. The product could not be crystallized nor could its picrate and perchlorate. It behaved as an aldehyde in that it gave a precipitate with Brady's reagent and restored the color to Schiff's reagent.

The gummy product of the above reaction (100 mg) was dissolved in 80% methanol (15 ml) and sodium borohydride (1.0 g) was added to the solution. After 2 hours the methanol was evaporated and a little water added to the residue which was extracted with ether. The extract was dried over sodium sulphate and the solvent distilled off. There was left a gum which was converted to a picrate that crystallized from methanol as large yellow prisms (0.8 g), m.p. 168–170°. Found: C, 55.39; H, 5.31. Calc. for $C_{19}H_{26}O_2N_2.C_6H_3O_7N_3$: C, 55.24; H, 5.38%. The base was regenerated from a solution of the picrate in methanol on a basic resin (Amberlite IRA 400). It consisted of a colorless glass which could not be crystallized, $[\alpha]_D^{22}$ +41.9° (*c*, 0.99 in ethanol). Found: C, 72.73; H, 8.34; N, 8.81. Calc. for $C_{19}H_{26}O_2N_2$: C, 72.58; H, 8.34; N, 8.91%.

Isomerization of Rhyncophyllol

A sample (2) of crystalline rhyncophyllol (100 mg) was refluxed in pyridine (25 ml) overnight. The solvent was then evaporated leaving a gum which failed to crystallize. It was dissolved in methanol and converted to the picrate which separated in yellow prisms (135 mg), m.p. 168–169°, undepressed in admixture with the picrate of isorhyncophyllol obtained by reduction of isorhyncophyllal.

Wolff–Kishner Reduction of Isorhyncophyllal

Amorphous isorhyncophyllal (400 mg) was reduced by the Huang–Minlon modification of the Wolff–Kishner reaction. The reaction mixture, after being cooled, was acidified with *N* hydrochloric acid and extracted with ether. The aqueous layer was alkalized with sodium carbonate and extracted repeatedly with ether. The extract from the basic solution was washed with water, dried over sodium sulphate, and evaporated to dryness. The residual stiff gum (205 mg) was distilled at 200° under 6.3 mm and obtained as a colorless glass which formed a picrate that crystallized from ethanol, m.p. 226–227°, either alone or in admixture with rhyncophyllane picrate (2). Found: C, 56.95; H, 5.26. Calc. for $C_{19}H_{26}ON_2.C_6H_3O_7N_3$: C, 56.92; H, 5.54%. The base, liberated on Amberlite IRA 400, had $[\alpha]_D^{24}$ +26.2° (*c*, 2.1 in ethanol) and its infrared absorption spectrum was identical with that of rhyncophyllane. As explained above, this compound should be renamed isorhyncophyllane.

N-Methylisorhyncophyllane

To a solution of isorhyncophyllane (60 mg) in methanol (5 ml) was added a methanolic solution of sodium methoxide (sodium, 50 mg, in methanol, 5 ml) and methyl iodide (1 ml). The mixture was refluxed on the water bath for 3 hours and then evaporated to dryness. The residue was extracted with ether, the extract washed with water, dried over sodium sulphate, and evaporated. A gummy residue was thus obtained which was dissolved in ethanol and converted to the picrate which separated as yellow prisms (50 mg), m.p. 182–184°. Found: C, 58.12; H, 5.86. Calc. for $C_{20}H_{28}ON_2.C_6H_3O_7N_3$: C, 57.66; H, 5.77%. The base liberated on Amberlite IRA 400 did not show any absorption bands in the NH—OH region of the infrared.

Mercuric Acetate Oxidation of Isorhyncophyllane

To a solution of isorhyncophyllane (400 mg) in 5% aqueous acetic acid (5 ml) was added mercuric acetate (2.4 g) dissolved in 5% aqueous acetic acid (10 ml) and the mixture heated on the steam bath. A precipitate of mercurous acetate started to appear after 15 minutes. The mixture was heated for 4 hours, cooled, and filtered. The dried mercurous acetate weighed 687 mg. The filtrate was boiled and treated with hydrogen sulphide until precipitation was complete. The mercuric sulphide was filtered off through supercel and the filtrate alkalized with excess sodium carbonate and extracted three times with chloroform. The extract was washed first with several portions of dilute hydrochloric acid then with water, dried over sodium sulphate, and evaporated. It left a neutral residue (180 mg) which could not be induced to crystallize and could not be distilled without some decomposition. The infrared spectrum of the product showed in addition to the oxindole carbonyl at 1705 cm^{-1} a strong band at 1625 cm^{-1} indicative of a six-membered lactam. Found: C, 70.99; H, 8.20. Calc. for $C_{19}H_{26}O_2N_2$: C, 72.58; H, 8.34%.

Reduction of Oxidation Product

Reduction of the oxidation product with lithium aluminum hydride in ether, in tetrahydrofuran, and in dioxane was attempted under the usual experimental conditions. Although the six-membered lactam carbonyl was reduced readily, the oxindole carbonyl could not be completely reduced. To a solution of lithium aluminum hydride (150 mg) in dry ether was added a solution of the oxidation product (98 mg) in dioxane (10 ml). The ether was evaporated off and the resulting slurry boiled under reflux for 24 hours under dry nitrogen. After the reaction mixture was cooled, the excess lithium aluminum hydride

was decomposed with water and the solution extracted with chloroform. The extract was washed with water and then with *N* sulphuric acid. The acid solution turned violet. It was made basic with aqueous sodium carbonate and extracted with chloroform. The extract yielded a gum (29 mg) which could not be crystallized and from which no crystalline salt could be obtained. The infrared spectrum of the product still contained a weak oxindole carbonyl band, but the lactam carbonyl absorption had completely disappeared.

Isomitraphylline

Mitraphylline (325 mg) was refluxed in pyridine (25 ml) overnight. The solvent was evaporated and the residue dissolved in a small volume of ether. After cooling for several hours, mitraphylline, m.p. 265 (66 mg), had crystallized. The residue recovered from the mother liquor was converted into a picrate which crystallized from methanol as yellow prisms, m.p. 223° (dec.) (377 mg). Found: C, 54.31; H, 4.38. Calc. for $C_{21}H_{24}O_4N_2.C_6H_3O_7N_3$: C, 54.36; H, 4.56%. The base, regenerated from the picrate on Amberlite IRA 400 was soluble in ether. It could not be induced to crystallize. Found: C, 68.03; H, 6.60; N, 7.42. Calc. for $C_{21}H_{24}O_4N_2$: C, 68.46; H, 6.57; N, 7.60%. Infrared spectrum in chloroform: 3440, 1720(87), 1626(72), 1118(63), 1098(62) cm^{-1}.

Dehydrogenation of Isomitraphylline and Mitraphylline

(*a*) Isomitraphylline (110 mg) and mercuric acetate (400 mg) in 5% acetic acid (4 ml) was kept at 40–50°. Precipitation of mercurous acetate started after 2 minutes. After 7 hours the reaction mixture was worked up as described for isorhyncophylline, and the product was converted to perchlorate. Reduction of the perchlorate in 80% methanol with sodium borohydride produced a mixture which was separated into mitraphylline (2 mg) and isomitraphylline isolated as the picrate (20 mg), m.p. 223–227°, undepressed by admixture with an authentic sample.

(*b*) When mitraphylline (100 mg) and mercuric acetate in 5% acetic acid were kept at 40–50° for 7 hours, only a trace of mercurous acetate had precipitated.

Octahydroformosanine

Formosanine (25 mg) was hydrogenated in glacial acetic (10 ml) over Adams' catalyst (50 mg) at 27° and 756 mm. The hydrogen absorbed was 8.1 ml (calculated for four double bonds, 7.2 ml). The catalyst and solvent were removed and the residue alkalized with sodium carbonate solution and extracted with ether. The extract was dried over sodium sulphate, evaporated to dryness, and the residue crystallized from ether from which it separated as colorless cubes (12.5 mg), m.p. 110–120°. Found: C, 66.74; H, 8.59. Calc. for $C_{21}H_{32}O_4N_2$: C, 66.99; H, 8.57%. Infrared spectrum: 1745(80), 1707(92), 1100(81) cm^{-1}.

Equilibration of Formosanine

Formosanine (45 mg) was refluxed in pyridine overnight. The solvent was evaporated and the residue dissolved in a small volume of ether from which crystals of formosanine, m.p. 218° (8 mg), were deposited. The fraction more readily soluble in ether did not crystallize (uncarine-A) and was dissolved in carbon disulphide for determination of its infrared spectrum.

ACKNOWLEDGMENTS

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