ALKALOID STUDIES—XXXII[1]

STUDIES ON *SKYTANTHUS ACUTUS* MEYEN. THE STRUCTURE OF THE MONOTERPENOID ALKALOID SKYTANTHINE[2]

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Abstract—Investigation of a Chilean member of the *Apocynaceae* family, *Skytanthus acutus* Meyen, has led to the isolation of a new monoterpenoid alkaloid which we have named skytanthine. The isolation and structure elucidation of this alkaloid (VIII) is described.

OUR interest in indole alkaloids from Latin American *Apocynaceae* has led to the investigation of a Chilean member of this family, *Skytanthus acutus* Meyen. Somewhat surprisingly, we did not encounter any indolic material but, on the other hand, there was isolated a new alkaloid which has been named "Skytanthine".

Skytanthine is an optically active liquid alkaloid (b.p. 54°/1·5 mm, $[\alpha]_D$ +42°) of the elementary composition $C_{11}H_{21}N$ and possesses two C-methyl functions and one N-methyl group. The infrared spectrum was of a simple nature with no characteristic absorption for any functional group and there was no absorption in the ultraviolet region. The alkaloid readily formed a crystalline picrate, m.p. 135–136° and a crystalline methiodide, m.p. 296–298°. The base did not take up any hydrogen, even under rather drastic conditions, and it was concluded that a bicyclic skeleton must be present.

In order to determine the nature of the nitrogen atom, skytanthine methiodide was subjected to Hofmann degradation. The isolation of a basic methine (III), $C_{12}H_{23}N$, provided conclusive evidence that the nitrogen atom must be part of a ring and the infrared spectrum of this Hofmann product, with bands at 6·05 μ and 11·30 μ, strongly suggested the presence of a terminal double bond. Confirmation was obtained when ozonolysis yielded formaldehyde, identified as the 2,4-dinitrophenylhydrazone derivative. The Hofmann product very readily absorbed one mole of hydrogen and the infrared spectrum of the reduction product showed, as expected, a complete absence of the terminal double bond absorption bands.

[1] Paper XXXI: E. Mosettig, P. Quitt, U. Beglinger, J. A. Waters, H. Vorbrueggen and C. Djerassi, *J. Amer. Chem. Soc.* **83** in press (1961).

[2] A preliminary communication of this work has appeared; C. Djerassi, J. P. Kutney, M. Shamma, J. N. Shoolery and L. F. Johnson, *Chem. & Ind.* 210 (1961).

Careful examination of the ozonolysis reaction mixture provided the other fragment which still possessed the remaining carbon atoms of the alkaloid. This substance, with strong carbonyl absorption at 5·83 μ in the infrared spectrum, was shown to be a dimethylamino ketone and since it exhibited a positive iodoform reaction, it was assumed to be a methyl ketone. The analytical results of this liquid as well as of its crystalline methiodide, m.p. 151–152°, indicated a molecular formula of $C_{11}H_{21}NO$ (IV).

To deduce the nature of the other carbon atom attached to the ketone grouping of the ozonolysis product, we subjected the methyl ketone to a peroxytrifluoroacetic acid oxidation[3] and obtained a liquid (V), $C_{11}H_{21}NO_2$, which possessed the typical infrared absorption (5·74 μ and 8·0 μ) of an acetate. This substance could be readily saponified to a secondary alcohol (VI), $C_9H_{19}NO$, which upon oxidation with chromium trioxide in acetone solution[4] yielded a ketone (VII) which possessed a strong carbonyl absorption (5·73 μ) in the infrared, typical of a cyclopentanone. The isolation of this five-membered ring ketone was the key to the determination of the nature of the remaining carbon atoms of skytanthine. Thus, it is clear that the production of a ketone in the oxidation reaction must proceed from a secondary alcohol function attached to a cyclopentane skeleton, and combined with the information derived from the previous reactions, leads to partial structure (I) for the alkaloid.
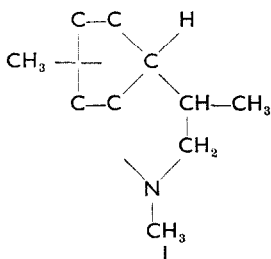

It is to be noted that this partial structure contains ten of the eleven carbon atoms of skytanthine. The remaining problem at hand concerned the location of the last carbon atom and the determination of the size of the second ring. These points were established from the information obtained when skytanthine was subjected to dehydrogenation using palladium black at 280° for 45 min. The product, an optically inactive pyridine, yielded a crystalline picrate, m.p. 139–140°, and under the conditions of a modified Kuhn–Roth oxidation,[5] yielded only acetic acid. It was quite certain, therefore, that any alkyl groups attached to the pyridine nucleus must be methyl groups or branched-chain substituents. The analytical figures of the picrate did not differentiate rigorously between the formulae $C_{10}H_{13}N$ and $C_{10}H_{15}N$, but nuclear magnetic resonance (NMR) spectroscopy did establish the correct empirical formula and the substitution pattern of the pyridine. The NMR spectrum[2] was in complete accord with the formula, $C_{10}H_{13}N$, and the signals present in the spectrum indicated that the pyridine was substituted *unsymmetrically* in the 3, 4 and 5 positions. Specific assignments[2] of the signals lead to two structures for the unknown pyridine, namely II or the alternate expression with the methyl group attached at the starred position.

[3] W. D. Emmons and G. B. Lucas, *J. Amer. Chem. Soc.* **77**, 2287 (1955).
[4] K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.* 39 (1946).
[5] H. Bickel, H. Schmid and P. Karrer, *Helv. Chim. Acta* **38**, 649 (1955).

It is important to note that the pyridine in question encompasses all of the carbon atoms of the parent alkaloid with the exception of the N-methyl function. Consequently, conclusive proof of its structure, along with the other data previously obtained, will decide between expressions VIII and IX for skytanthine. Of these, the former appeared more plausible on biogenetic grounds, since it is an example of a rather common monoterpenoid skeleton[6] following the isoprene rule.
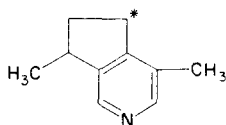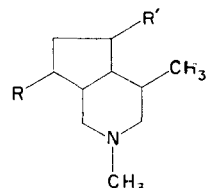

II
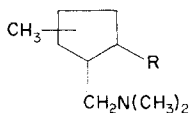
III , R = C(=CH_2)CH_3
IV , R = C(=O)CH_3
V , R = OCOCH_3
VI , R = OH
VII , R = O

VIII , R = CH_3; R' = H
IX , R = H; R' = CH_3

Indeed our preference for VIII and, therefore, likewise for structure II, was supported by the fact that such an arrangement of atoms had been found recently[7] in the optically active alkaloid actinidine to which the Japanese workers attributed structure II. Comparison (mixture melting point, infrared and NMR) of our pyridine (II) picrate with a sample of synthetic racemic actinidine picrate[8] demonstrated the identity of the two substances. It follows therefore that skytanthine must possess structure VIII[9] and that it represents a member of the very rare class of monoterpenoid alkaloids.[7,10]

EXPERIMENTAL

All melting points were taken on a Kofler melting point apparatus and are uncorrected. The infrared spectra on the pure liquids were taken as liquid films unless stated otherwise. The ultraviolet spectra were measured in 95% ethyl alcohol.

Isolation scheme

(a) *Soxhlet procedure at elevated temperature.* The dried and well-ground bark (5 kg) of the plant *Skytanthus acutus* was exhaustively extracted in a Soxhlet extractor with boiling methanol for five days. The total alcoholic extract (ca. 10 l.) was concentrated *in vacuo* on a steam bath to a volume of 1·5 l. To this concentrated solution, water (3 l.) and glacial acetic acid (500 ml) were added and the turbid mixture was then repeatedly extracted with hexane to remove colored, tarry materials.

The acid aqueous layer was extracted first with benzene. The benzene layer was washed with 10% aqueous acetic acid and the washings combined with the main aqueous layer. The benzene solution was then washed with 5% ammonium hydroxide, with water and then dried over anhydrous sodium sulfate. Removal of the solvent *in vacuo* gave 4·65 g of oily materials.

The acid aqueous layer was now extracted with chloroform in the same manner as above. Removal of the solvent *in vacuo* gave a crude, oily material (14 g).

[6] For numerous references, see C. Djerassi, T. Nakano, A. N. James, L. H. Zalkow, E. J. Eisenbraun and J. N. Shoolery, *J. Org. Chem.* **26**, 1192 (1961).
[7] T. Sakan, A. Fujino, F. Murai, Y. Butsugan and A. Suzui, *Bull. Chem. Soc. Japan* **32**, 315, 1155 (1959).
[8] We are very grateful to Professor T. Sakan, Osaka City University, for a gift of this substance.
[9] G. C. Casinovi, J. A. Garbarino and G. B. Marini-Bettolo, *Chem. & Ind.* 253 (1961) independently (see ref. 2) reached the same conclusion.
[10] K. Wiesner, Z. Valenta, B. S. Hurlbert, F. Bicklehaupt and L. R. Fowler, *J. Amer. Chem. Soc.* **80**, 1521 (1958).

The acid aqueous layer was then adjusted to pH 7 by the addition of ammonium hydroxide and the resulting mixture was extracted with chloroform. The chloroform extract, after washing with water, was dried over anhydrous sodium sulfate. Removal of the solvent *in vacuo* gave material (9 g), most of which was liquid and had a strong amine-like odor.

The aqueous layer was finally adjusted to pH 10–12 and extracted repeatedly with chloroform. Removal of solvent yielded basic, oily material (3·8 g). No indoles were encountered after careful chromatography of all of these fractions.

(b) *Percolation procedure at room temperature.* Dried and ground total plant (4 kg) was soaked in a methanol–10% acetic acid solution. The moist plant material was then packed into a large chromatography column and a methanol–10% acetic acid solution was percolated through it for several days until the solution was essentially colorless. A total of 30 l. of solvent was collected during the percolation. The extracts were concentrated at 70° *in vacuo* to yield 1·5 l. of a viscous residual oil. This oil was dissolved in 1·5 l. of water to which a minimum amount of solvent had been added to maintain a homogeneous solution. This dark solution was made strongly acidic with concentrated hydrochloric acid and then extracted repeatedly with ethyl ether until the extracts were essentially colorless. This ethereal extract, which contained the nonbasic materials, was not investigated further at this time.

The acidic layer was made alkaline by the addition of sodium hydroxide pellets and the alkaline solution was extracted thoroughly with many portions of ether (a total of about 16 l. was used). The ether extract was dried over anhydrous magnesium sulfate and the solvent evaporated to yield a brown residual oil. Distillation (see below) of this oil provided the alkaloid in a yield of 0·03% based on dry plant.

Characterization of skytanthine

The residual oil from the basic fraction of the extraction was distilled at 100°/4 mm to yield a colorless liquid with a very strong amine-like odor. A part of this material was carefully fractionated into several fractions but the infrared spectra of each of these fractions were indistinguishable, thereby indicating that the alkaloid was homogeneous. Another careful fractionation gave pure skytanthine (VIII), b.p. 54°/1·5 mm, n_D^{27} 1·4762, $[\alpha]_D$ –42° (CHCl$_3$). (Found: C, 78·82; H, 12·58; N, 8·26; C–CH$_3$, 16·64; N–CH$_3$, 8·27. C$_{11}$H$_{21}$N requires: C, 78·97; H, 12·65; N, 8·37; 2C–CH$_3$, 17·96; N–CH$_3$, 8·98%). A *picrate* of skytanthine was obtained from ether, m.p. 124–130° and after three recrystallizations from methanol afforded yellow crystals, m.p. 135–136°. (Found: C, 51·42; H, 5·96; N, 14·09; O, 28·40. C$_{17}$H$_{24}$O$_7$N$_4$ requires: C, 51·51; H, 6·10; N, 14·14; O, 28·26%).

A crystalline *methiodide* was also prepared by the addition of an excess of methyl iodide to an ethanolic solution of the alkaloid. The initial crop of crystals, m.p. 295–300° (dec.) was recrystallized from ethanol and an analytical sample, m.p. 296–298° (dec.) was obtained. (Found: C, 47·23; H, 7·72; I, 40·37. C$_{12}$H$_{21}$NI requires: C, 46·60; H, 7·82; N, 4·56; I, 40·37%).

Hofmann degradation of skytanthine

Freshly prepared silver oxide from silver nitrate (500 mg) and sodium hydroxide was washed free of excess hydroxide and then added slowly, over a period of 15 min, to skytanthine methiodide (250 mg) dissolved in water (18 ml). The mixture was stirred vigorously for 2 hr and then allowed to stand overnight. The precipitate of silver oxide and silver iodide was filtered and most of the solvent was distilled off until a small volume of solution remained. This solution was transferred to a microdistillation apparatus and distilled at atmospheric pressure. The distillate was saturated with potassium hydroxide and extracted with ethyl ether. The ether layer was first dried over anhydrous sodium sulfate and then the solvent was carefully removed through a fractionating column. The small amount of product which remained was taken up in acetone (3 ml) and to this mixture, methyl iodide (0·5 ml) was added. The mixture was allowed to stand for 0·5 hr during which time, white crystals separated from the solution. Evaporation of the solvent gave colorless crystals (155 mg) m.p. 135–150°. One recrystallization from methanol-benzene gave a first crop (10 mg) of crystals, m.p. 293–296° which proved to be unreacted skytanthine methiodide. A second crop (110 mg) was obtained from the same solvent and after recrystallization from acetone melted at 145–147°, $[\alpha]_D$ +29° (ethanol). (Found: C, 47·92; H, 8·23; N, 4·28; I, 38·94. C$_{13}$N$_{26}$NI requires: C, 48·30; H, 8·42; N, 4·36; I, 39·28%).

Subsequent experiments using larger quantities (0·5 g) of skytanthine methiodide yielded an

analytical sample of the liquid methine III, b.p. 170°, $[\alpha]_D$ +30° (methanol), λ_{max}^{capill} 6·05 and 11·30 μ. (Found: C, 79·57; H, 12·50; N, 8·23. $C_{12}H_{23}N$ requires: C, 79·49; H, 12·79; N, 7·73%).

The freshly distilled methine (15 mg) was hydrogenated, using platinum oxide catalyst (15 mg) and glacial acetic acid (5 ml) as the solvent. A very rapid uptake of hydrogen was observed and 0·9 mole of hydrogen was taken up in 3 min after which time the absorption of hydrogen ceased. The infrared spectrum indicated a complete disappearance of the peaks at 6·05 and 11·30 μ.

Ozonolysis of methine (III)

Freshly distilled methine (150 mg) was dissolved in glacial acetic acid (10 ml) and the mixture ozonized at room temperature for 15 min. The ozonide was transferred directly to a flask containing a solution of ferrous sulfate (1 g) in water (10 ml) and the reaction mixture was then stirred at room temperature for 0·5 hr. It was finally heated for 15 min and then immediately steam distilled into a solution of 2,4-dinitrophenylhydrazine reagent consisting of 2,4-dinitrophenylhydrazine (0·59 g), conc. sulphuric acid (10 ml), water (40 ml). The 2,4-dinitrophenylhydrazine solution immediately became cloudy and a precipitate soon formed. The phenylhydrazone was filtered off, dried, dissolved in a small amount of benzene and chromatographed on Fischer activated alumina. Concentration of the benzene fractions yielded a 2,4-dinitrophenylhydrazone (40 mg), m.p. 162–165°, subsequently shown to be formaldehyde 2,4-dinitrophenylhydrazone by mixture melting point and infrared comparison.

To confirm that only one aldehyde component was, in fact, present in the ozonolysis distillate, a paper chromatogram was also run on the 2,4-dinitrophenylhydrazone derivative. The chromatogram (using cyclohexane-dimethylformamide as the moving phase and methanol-dimethylformamide as the stationary phase) indicated only one spot which was identified as the derivative of formaldehyde by comparison with an authentic specimen.

The acidic residue remaining after the steam distillation, was made basic by the addition of sodium hydroxide and this basic aqueous layer was extracted several times with ether. The ether extract was dried over anhydrous sodium sulfate and the solvent carefully evaporated to yield a yellowish liquid product (112 mg). This product was distilled at 100°/4·5 mm to yield an analytical sample of the *methyl ketone* IV, $[\alpha]_D$ +34° (methanol), λ_{max}^{capill} 5·83 μ. (Found: C, 72·27; H, 11·41; O, 8·92. $C_{11}H_{21}NO$ requires: C, 72·08; H, 11·55; O, 8·73%).

A crystalline *methiodide* of IV was readily prepared by the addition of methyl iodide to an alcoholic solution of the compound. Recrystallization of the solid from ethanol yielded the pure methiodide, m.p. 151–152°. (Found: C, 44·10; H, 7·57. $C_{12}H_{24}NIO$ requires: C, 44·31; H, 7·38%).

Peroxytrifluoroacetic acid oxidation of methyl ketone (IV)

The preparation of peroxytrifluoroacetic acid was carried out by the addition of trifluoroacetic anhydride (2 ml) to a flask containing 90% hydrogen peroxide (0·5 ml) and methylene chloride (10 ml). This mixture, was stirred at room temperature for several min and then added dropwise, over a period of 5 min to a stirred mixture of the methyl ketone (IV) (100 mg) and sodium dihydrogen phosphate (0·5 g) in methylene chloride (20 ml). After completion of the addition of peracid, the reaction mixture was stirred at room temperature for 20 min and finally heated under reflux for a further 20 min. Upon cooling, a concentrated solution of sodium sulfite was added dropwise, with stirring, until no further reaction was evident. Water (10 ml) was then added and stirring was continued for 0·5 hr to ensure complete hydrolysis of any excess anhydride. The reaction mixture was made alkaline by the addition of a solution of sodium hydroxide and this was then extracted with methylene chloride (100 ml). The methylene chloride extract was dried over anhydrous magnesium sulfate, and the solvent was carefully fractionated to yield a light yellow liquid product. Distillation of this liquid at 110°/7 mm yielded the colorless acetate V (73 mg), $[\alpha]_D$ +20 (methanol), λ_{max}^{capill} 5·74, 8·0 μ. (Found: C, 66·22; H, 10·21; N, 6·84. $C_{11}H_{21}NO_2$ requires: C, 66·29; H, 10·62; N, 7·03%).

Saponification of acetate (V)

The ester (50 mg) from the peracid reaction was treated with 20% aqueous sodium hydroxide (1 ml) and several drops of methanol to increase the solubility and the mixture was refluxed for 0·5 hr. The cooled reaction mixture was extracted exhaustively with ether and the ethereal extract was dried over anhydrous sodium sulfate. Careful fractionation of the solvent yielded a yellowish liquid

(25 mg) which upon distillation at $100°/7$ mm provided a pure sample of the *alcohol* VI, $[\alpha]_D$ 18 (methanol), λ_{max}^{capill} 2·95 μ. (Found: C, 68·40; H, 12·27. $C_9H_{19}NO$ requires: C, 68·74; H, 12·18 %).

Chromium trioxide oxidation of alcohol (VI)

The alcohol (60 mg) from the above saponification was dissolved in acetone (2 ml), cooled to 15 and then treated with Jones reagent[4] (1 ml). After standing at room temperature for 5 min, small amounts of crystalline salts began to separate. The reaction mixture was then treated with water (5 drops) to dissolve the salts and the mixture allowed to stand for a further 20 min. The mixture was diluted with water (50 ml) and made basic by the addition of sodium hydroxide. Continuous ether extraction for 3 hr yielded, after drying and evaporation of solvent, a liquid, which was distilled at $100°/8$ mm to yield the pure colorless *ketone* VII, (48 mg), $[\alpha]_D$ – 9° (methanol), λ_{max}^{capill} 5·73 μ. (Found: C, 69·41; H, 10·83. $C_9H_{17}NO$ requires: C, 69·63; H, 11·04 %).

Palladium dehydrogenation of skytanthine

Freshly distilled skytanthine (0·5 g) and palladium black (100 mg) were placed in a small round bottom flask. The flask was attached to a water-cooled condenser which, in turn, was attached to a trap cooled in ice. The flask was then immersed in a bath and the temperature of the bath was gradually raised to 280° and maintained at this temperature for 45 min. The reaction mixture was then cooled and extracted with ether. The ether extract was dried over anhydrous sodium sulfate and the solvent removed to yield a crude brownish liquid product (450 mg). The liquid was then treated with a saturated solution of picric acid in 95 % ethanol and the mixture was heated to boiling. Upon cooling, dark yellow crystals separated and these were filtered off. To remove any dark material, the crystals were taken up in methyl alcohol and decolorized several times with Norit. The clear yellow solution which resulted after filtration was concentrated and a new crop of crystals was obtained. Several recrystallizations from methyl alcohol yielded a pure picrate (500 mg), m.p. 139–140°, whose melting point was undepressed upon admixture with racemic actinidine (II) picrate.[7,8] (Found: C, 50·95; H, 4·44; N, 14·51. $C_{15}H_{18}N_4O_7$ requires: C, 50·79; H, 4·80; N, 14·81 %: $C_{16}H_{18}N_4O_7$ requires: C, 51·06; H, 4·29; N, 14·89 %).

To recover the free base, the above picrate (500 mg) was treated with an aqueous saturated solution of lithium hydroxide (10 ml) and the alkaline solution was extracted with several small portions of ether. The ether extract was dried over anhydrous magnesium sulfate, the solvent evaporated and the residual oil was distilled to yield racemic *actinidine* (II) (175 mg), λ_{max} 263 mμ, log ϵ 3·15, whose infrared and NMR spectra were identical with those of synthetic material.[7,8]

Micro Kuhn–Roth oxidation[5]

Before the pyridine isolated from the palladium dehydrogenation of skytanthine was oxidized, several model compounds and blanks were determined. The compound, 3-methyl-4-n-butylpyridine, yielded butyric, propionic and acetic acids whereas the substance, 3-methyl-4-sec-butylpyridine yielded propionic and acetic acids.

Under these conditions, the pyridine (II) from the dehydrogenation of skytanthine produced only acetic acid.