THE STRUCTURE OF INDACONITINE[1]

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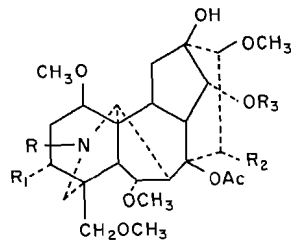
ABSTRACT

The alkaloid indaconitine has been converted into delphinine by replacement of a secondary hydroxyl by hydrogen and substitution of an imino-methyl for the imino-ethyl group originally present. This correlation rigorously establishes the structure of indaconitine and its absolute configuration. The product of the saponification of indaconitine, pseudaconine, is identical with the product of the saponification of pseudaconitine, and this last alkaloid has been correlated previously with aconitine. It follows that the secondary hydroxyl which was removed in the conversion of indaconitine to delphinine has the same configuration as in aconitine.

Indaconitine ($C_{34}H_{47}O_{10}N$) has been isolated from *Aconitum chasmanthum* Stapf by Dunstan and Andrews (1) nearly sixty years ago. According to these authors, it melts at 202–203°, has $[\alpha]_D$ +18.3° (EtOH), and forms crystalline salts. Further, on acid hydrolysis it yields acetic acid and benzoylpseudaconine, while on alkaline hydrolysis it produces acetic acid, benzoic acid, and pseudaconine ($C_{25}H_{41}O_8N$) which still contains the four methoxyl groups present in indaconitine. The alkamine, pseudaconine, is identical with that produced in the alkaline hydrolysis of pseudaconitine ($C_{36}H_{51}O_{12}N$). When heated above its melting point, indaconitine loses one mole of acetic acid and gives rise to pyroindaconitine ($C_{32}H_{43}O_8N$). It has also been established that indaconitine contains an imino-ethyl group (2).

In its behavior on hydrolysis and on pyrolysis indaconitine resembles aconitine I (R = Et; R_1 = R_2 = OH; R_3 = C_6H_5CO) and delphinine I (R = Me; R_1 = R_2 = H; R_3 = C_6H_5CO).



I

Since the empirical formula of indaconitine differs from that of aconitine only in containing one oxygen less, it has been assumed as a working hypothesis that indaconitine possessed the same carbon–nitrogen skeleton as aconitine (3, 4) and that it lacked one of the three hydroxyls present in the latter. The first step in the investigation, which has been the subject of a preliminary communication (5), was to determine which of the hydroxyls present in aconitine was absent in indaconitine.

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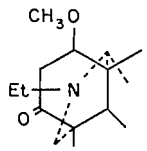
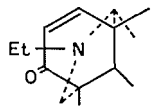
Aconitine is known to be oxidized by chromic oxide to the ketone, aconitinone (partial formula II), which readily loses the elements of methanol to form the $\alpha\beta$-unsaturated



II

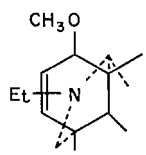


III

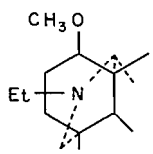
ketone, aconitoline III (6). Indaconitine has been found to undergo a similar oxidation with the formation of indaconitoline ($C_{33}H_{45}O_9N$), which is an $\alpha\beta$-unsaturated six-membered cyclic ketone as suggested by an absorption band at 1 675 cm^{-1} (CO) in its infrared spectrum and one at 710 cm^{-1} indicative of a double bond. Hence, like aconitine, indaconitine seems to contain a secondary hydroxyl group at R_1.

In the pyrolysis of aconitine (in which R_2 = OH) the acetoxy group is eliminated with a neighboring hydrogen, and there results the formation of an enol that tautomerizes to a ketone (7). On the other hand, in the pyrolysis of delphinine (in which R_2 = H) a similar elimination takes place resulting in the formation of an olefinic bond (8, 9). Pyroindaconitine, the product of the pyrolysis of indaconitine, was transesterified to pyropseudaconine, which showed no carbonyl absorption in the infrared but did show an absorption band at 1 620 cm^{-1} indicative of a double bond. It has been shown previously (10) that the double bond in pyropseudaconine can be isomerized and can be hydrogenated just like the double bond in pyrodelphonine. It can thus be concluded that it is the R_2 hydroxyl of aconitine that is absent in indaconitine, and that the structure of the latter must be represented by I (R = Et; R_1 = OH; R_2 = H; R_3 = C_6H_5CO). In order to prove or disprove this conclusion an attempt was made to convert indaconitine into delphinine by removal of the R_1 hydroxyl and replacement of the *N*-ethyl group by an *N*-methyl group.

Indaconitine when refluxed with thionyl chloride lost the elements of water and was converted into anhydroindaconitine, $C_{34}H_{45}O_9N$ (IV), which was purified as its perchlorate. Catalytic hydrogenation of anhydroindaconitine in absolute ethanol over platinic oxide gave rise to deoxyindaconitine (V) which, when refluxed in 3% acetic acid with



IV



V

mercuric acetate, lost the imino-ethyl group. The amorphous secondary base, *N*-desethyl-deoxyindaconitine, thus produced had an n.m.r. spectrum that contained no ethyl group signal. That no other change had taken place in the course of the last reaction than the removal of the imino-ethyl group was ascertained by conversion of a small quantity of the product to deoxyindaconitine via the action of ethyl iodide.

Refluxing *N*-desethyl-deoxyindaconitine for 1 h with methyl iodide gave *N*-methyl-*N*-desethyl-deoxyindaconitine, m.p. 188–192°, $[\alpha]_D^{27}$ +26° (EtOH). The recorded physical

constants of delphinine are $[\alpha]_D^{25}$ +25° (EtOH) and m.p. 198–200°. The melting point was not altered by mixture of the product with an authentic sample of delphinine. The behavior of the product on a chromatoplate was identical with that of delphinine, and a mixture of the two gave only one spot. The infrared absorption spectra of both were superimposable, and the X-ray powder patterns were identical.

The conversion of indaconitine into delphinine confirms the correctness of the assumed structure of indaconitine I (R = Et; R_1 = OH; R_2 = H; R_3 = C_6H_5CO). Since the reactions involved in the conversion do not disturb the asymmetric centers of the molecule except for the removal of the R_1 hydroxyl, the stereochemical arrangement present in delphinine must also be present in indaconitine. Furthermore, since pseudaconitine has been correlated with aconitine (10), and the hydrolytic alkamine derived from indaconitine is identical with that derived from pseudaconitine, it follows that the stereochemistry of the R_1 hydroxyl of indaconitine is the same as that of the R_1 hydroxyl of aconitine.

Consequently, the conversion of indaconitine into delphinine not only proves the structure of indaconitine, but also establishes its absolute configuration to be the same as that of aconitine and delphinine. The relative positions and the configurations of the two ester groups in indaconitine which are derived from the correlation are also confirmed by the n.m.r. characteristics of the alkaloid (cf. ref. 11).

EXPERIMENTAL

In all column chromatographic separations, Woelm neutral alumina was used. Melting points were determined on a Kofler hot stage. The n.m.r. spectra were obtained on a Varian A-60 instrument.

Indaconitine

The indaconitine used in this investigation was isolated from the root of *Aconitum chasmanthum* Stapf.[3] It was purified by chromatography in ether solution over grade III alumina, and eluted with benzene–chloroform (4:1). The base recovered from the second fraction of the eluate was crystallized several times from ether, from which it separated as colorless hexagonal plates, m.p. 193–195° (decomp.), $[\alpha]_D^{25}$ +19.2° (*c*, 0.78 in EtOH).

Anal. Calcd. for $C_{34}H_{47}O_{10}N$: C, 64.86; H, 7.47. Found: C, 64.63; H, 7.40%.

The n.m.r. spectrum indicates the presence of four methoxyl groups, one *O*-acetyl, and one imino-ethyl.

Indaconitoline

Indaconitine (208 mg) was dissolved in acetone (10 ml). The solution was cooled to 5° and added to a solution of chromic oxide (210 mg) in acetone (10 ml) also previously cooled to 5°. The reaction mixture was set aside at room temperature for 2 d, by which time it had formed a bulky brown deposit. The mixture was evaporated to dryness, water was added to the residue, and a stream of sulfur dioxide was bubbled through until a clear solution was obtained (15 min). The solution was made basic (pH 8) with solid potassium carbonate and repeatedly extracted with chloroform. The combined extract was washed with water, dried over sodium sulfate, and evaporated to dryness. A residual colorless glass was left which was dissolved in ether; the solution was filtered and evaporated to dryness. The ether-soluble residue was dissolved in a 1:1 benzene–hexane mixture and chromatographed on grade III alumina. The chromatogram was eluted first with 1:1 benzene–hexane, then with benzene, and finally with chloroform. The chloroform eluate yielded the bulk of the material which was further purified by large scale thin-layer chromatography on silica gel, with benzene–cyclohexane–ethylamine (4.5:4.5:1) as developing solvent. The top main band was scraped off and extracted with chloroform, and the product left after evaporation of the chloroform was crystallized from ether – petroleum ether. The colorless indaconitoline (at a slow rate of heating) melted at 208–211° (decomp.).

Anal. Calcd. for $C_{33}H_{41}O_9N$: C, 66.54; H, 6.94; N, 2.35. Found: C, 66.41; H, 6.88; N, 2.43%.

Infrared spectrum analysis: ν, 1 725 cm^{-1} (ester carbonyl), 1 675 cm^{-1} ($\alpha\beta$-unsaturated six-membered cyclic ketone), 1 225 cm^{-1} (acetyl ester), 710 cm^{-1} (C=C).

Pyroindaconitine

Indaconitine (710 mg) was introduced into a distillation bulb which was then evacuated to 5 mm and heated in an air bath at 200–205° for 4 min. Evolution of gas took place. After cooling, the residue in the bulb was dissolved in ether, chromatographed in grade IV alumina, and eluted with ether. The residue (465 mg)

[3]*Obtained through the courtesy of Mr. K. L. Handa, Regional Research Laboratory, Jammu, India, to whom we express our gratitude.*

from the ether eluate was a colorless gum that could not be induced to crystallize. In the n.m.r. the acetyl protons signal was no longer present.

Pyroindaconitine (465 mg) was dissolved in absolute ethanol (30 ml) and a few mg of sodium was added to the solution which was left at room temperature for 2.5 h. The solution was evaporated to dryness, and the residue was dissolved in water and extracted with petroleum ether to remove the ethyl benzoate. The aqueous liquor was then extracted with chloroform, the extract was evaporated to dryness, and the residue was crystallized from ether. This product partially melted at 89–90°, resolidified, and melted at 157–167° (decomp.). In admixture with an authentic sample of pyropseudaconine (12), the behavior on melting was identical. The infrared spectrum contained a band at 1 620 cm^{-1} indicative of a double bond, but contained no carbonyl absorption. Pyropseudaconine readily forms a perchlorate which crystallizes from acetone–ether as colorless clusters, m.p. 215–225° (decomp.).

Anhydroindaconitine

Indaconitine (500 mg) was dissolved in thionyl chloride (previously distilled from quinoline) (5 ml), and the solution was refluxed for 4 h. The thionyl chloride was distilled off *in vacuo* and the residue (470 mg) was dissolved in methanol. The methanolic solution was evaporated to dryness, and the residue was dissolved in water, made basic (pH 8) with sodium carbonate, and extracted with three portions of chloroform. The combined extract was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue from the extract was dissolved in benzene–chloroform (2:1), chromatographed over grade III alumina, and eluted with the same solvent. Fractions of 50 ml were collected. Fractions 2 to 11 were combined and the residue left after evaporation was crystallized from chloroform. The crystalline base was converted to the perchlorate, which crystallized from methanol–ether as colorless prisms, m.p. 190–203° (decomp.), $[\alpha]_D^{27}$ +31° (*c*, 0.5 in EtOH).

Anal. Calcd. for $C_{34}H_{45}O_9N \cdot HClO_4$: C, 57.45; H, 6.53. Found: C, 57.32; H, 6.51%.

Deoxyindaconitine

Anhydroindaconitine (720 mg) recovered from the perchlorate was dissolved in 95% ethanol and hydrogenated catalytically over platinum oxide. The product, worked up as usual, was dissolved in methylene chloride, chromatographed on grade III alumina, and eluted with methylene chloride. The eluate yielded 520 mg of benzene-soluble substance which was crystallized from hexane, m.p. 175–180° (decomp.), $[\alpha]_D^{27}$ +14° (*c*, 0.6 in EtOH).

Anal. Calcd. for $C_{34}H_{47}O_9N$: C, 66.54; H, 7.72; N, 2.28. Found: C, 66.69; H, 7.55; N, 2.40%.

The perchlorate of the base, which separated from methanol–ether as large colorless crystals, decomposed at 177–183°.

Anal. Calcd. for $C_{34}H_{47}O_9N \cdot HClO_4$: C, 57.18; H, 6.77. Found: C, 56.89; H, 6.52%.

N-Desethyl-deoxyindaconitine

Deoxyindaconitine (95 mg) was dissolved in 3% acetic acid (25 ml) and the solution warmed on the steam bath. Mercuric acetate (500 mg) was added, and the solution was kept on the steam bath for 8 h and allowed to stand overnight at room temperature. The reaction mixture was filtered and the filtrate extracted with three portions of chloroform. The combined extract was washed with aqueous sodium bicarbonate and with water, dried, and evaporated to dryness. The residue (56 mg) was dissolved in benzene–hexane (1:1) and chromatographed on grade III alumina. The column was eluted first with benzene–hexane, then in succession with benzene, 10% methylene chloride in benzene, 1:1 methylene chloride – benzene, and methylene chloride. The last fraction eluted with methylene chloride yielded only a trace of product, which no longer contained starting material. The elution was then continued with chloroform, which yielded 32 mg of product. This product, which could not be induced to crystallize, showed no signal indicative of the *N*-ethyl group in the n.m.r. A sample of *N*-desethyl-deoxyindaconitine, by treatment with ethyl iodide in methanol, was converted back to deoxyindaconitine, identical with the starting material.

N-Methyl-N-desethyl-deoxyindaconitine

N-Desethyl-deoxyindaconitine (50 mg) was dissolved in ether (5 ml) and a few drops of methyl iodide were added to the solution. A white precipitate formed immediately and more methyl iodide (3 ml) was added until the precipitate had dissolved. The solution was then refluxed on the steam bath for 1 h and subsequently evaporated to dryness. It left a residue which was dissolved in hot water. The filtered solution was cooled in ice, made alkaline with sodium carbonate, and extracted with three portions of chloroform. The combined extract was washed with water, dried over sodium sulfate, and evaporated to dryness *in vacuo*. The residue (50 mg) was dissolved in benzene–*n*-hexane (1:1) and chromatographed on grade III alumina. The column was eluted first with the same solvent, then with benzene, and finally with methylene chloride, and the eluate was collected in 30 ml fractions. The benzene eluate (fractions 5–7) yielded a crystalline product (14 mg) which after crystallization from *n*-hexane started to sinter at 180° and melted at 188–192°; $[\alpha]_D^{27}$ +26° (*c*, 0.6 in EtOH).

Anal. Calcd. for $C_{33}H_{45}O_9N$: C, 66.09; H, 7.56. Found: C, 66.30; H, 7.66%.

In admixture with an authentic sample of delphinine, it sintered at 184° and melted at 188–191°. The infrared spectra of the product and of delphinine were superimposable, and the X-ray powder patterns of the two bases were identical.

ACKNOWLEDGMENTS

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