would be expected to out-perform corresponding sodium salt and might even be preferable to KO^{t}Bu in base catalysed reactions.

Mild saponification of crude benzenesulfonate (Ic) furnished, in ca. 95% yield, the corresponding crude hydroxy acid (If) ($E^{1\%}_{1\,cm}$ 217 mμ = 144), which upon treatment with butoxide-DMSO, as usual, offered (II) in 77% yield, in contrast to 83% yield obtained with pure crystalline (If), m.p. 134-135°, $[\alpha]^{25}_D + 56.82°$ (ε_{max} 217 mμ = 9,100). Likewise, (Ib) on saponification provided (Ig), m.p. 135–136°, $[\alpha]^{25}_D + 55.26°$ (ε_{max} 225 mμ = 12,200), which was converted into (II) in 88% yield.

Zusammenfassung. Die Abspaltung von Sulfonsäuren aus 12α-Sulfonsäureestern von Steroiden verläuft besonders leicht mit Kalium-tert.-butylat in einem dipolaren aprotischen Lösungsmittel (z.B. Dimethylsulfoxid). Die Δ^{11}-Verbindungen werden in Ausbeuten bis zu 88% erhalten.

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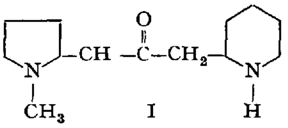
Research Laboratories, Canada Packers Limited, Toronto (Ontario, Canada), December 18, 1964.

Simulated Biosynthesis of Anahygrine

Anahygrine (I), a natural alkaloid, is shown to occur in the roots of *Withania somnifera* Dunal[1] together with other pyrrolidine and piperidine alkaloids. According to Robinson[2], anahygrine could be formed from one equivalent each of ornithine, lysine, and acetone. That these proposals are tenable has been demonstrated for a number of pyrrolidine and piperidine alkaloids in both in vivo and simulated biosynthetic systems with a variety of equivalents.



I

In this work the ornithine equivalent was N-methyl-2-hydroxypyrrolidine[3] (II) prepared by the reduction of N-methyl-2-pyrrolidone (IIa) with $LiAlH_4$. The alcoholate of II was used as such without purification. The lysine equivalent used was Δ^1-piperideine[4] (III). Acetone dicarboxylic acid (IV) served as the acetone equivalent.

The reaction mixture contained equimolar quantities of II (based upon IIa), III, and IV. Compound IV was added to a solution of II and III in 0.1 *N* NaOH and the pH was adjusted to 12 with the NaOH solution. The mixture, after maintenance at room temperature for 40 h, was acidified and heated on a steam bath to insure complete decarboxylation. The product was made alkaline and extracted with chloroform, and the dried extract was separated into the component alkaloids by a liquid-liquid distribution system.

Anahygrine was obtained and identified as the hydrochloride, m.p. 218–219°, and the picrate, m.p. 174° (reported 217° and 173–174°, respectively[1]). The infrared spectrum of the hydrochloride in KBr pellet was identical with a reference sample of natural and synthetic anahygrine hydrochloride.

In addition to anahygrine, four other alkaloids were separated from the reaction mixture. These were anaferine, cuscohygrine, isopelletierine and hygrine. All of these alkaloids, except hygrine, have been shown to occur in *Withania somnifera*[1].

Zusammenfassung. Kondensation von N-Methyl-2-hydroxypyrrolidin und Δ^1-Piperidein mit Acetondicarbonsäure liefert Anahygrin und vier verwandte Alkaloide.

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Pharmacognosy Research Laboratories, University of Connecticut, Storrs (USA), December 22, 1964.

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[5] Acknowledgment: This investigation was supported in part by grant GM-10070 from the National Institutes of Health.

Quantitative Investigation on the Possible Losses of Nucleic Acids from 'Freeze-Substituted' Tissue in the Course of Histological Procedure

In the course of investigations on the influence of temperature and media on the nitrogen and phosphorus content of tissues fixed by the 'freeze-substitution' method, it was found that when methanol was used for substitution there were considerable losses of tissue phosphorus[1,2]. It seemed interesting, therefore, to note whether during substitution, or in later stages of histochemical procedure, the considerable loss of phosphorus is not connected with nucleic acid loss. It was also of concern to know how great this loss was during the substitution itself, as well as from

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