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) ($E^{1\%}_{1cm}$ 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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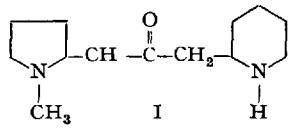
Simulated Biosynthesis of Anahygrine

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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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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the 'freeze-substituted' tissues in the course of subsequent water elution.

Materials and method. Male white Wistar rats, weighing 150 to 200 g, were used for the investigation. Figure 1 shows the details of the procedure used. For determination the nucleic acids were extracted by perchloric acid and their quantity was determined by extinction measurements in two wavelengths according to TSANEV and MARKOV[3]. Significance indices were calculated according to HILL[4].

Results. Table I presents dry mass values and nucleic acid contents in rat liver fixed in methanol at room temperature. These values are in agreement with the results of other authors[3,5], and were treated as control values. Table II shows the results obtained in the other three experimental groups. They are presented as percentages of dry mass and nucleic acid content of the control group. Nucleic acid content is given for wet and dry tissue weight. From the results presented in the Tables and in Figure 2, it may be seen that differences in extractability of nucleic acids are statistically significant only for the control group extracted by water and for the substituted group extracted by water as well. There is also a significant difference in the loss of dry mass between these two groups. The loss of dry mass from tissue substituted and extracted by water in relation to the control material treated in a similar way is also significantly different (Figure 2).

Discussion. In substituted material the dry mass loss was as high as 11% and was almost as high in tissue after normal fixation and water extraction. On the other hand, the mean dry mass loss of material substituted and water-extracted was bigger, reaching a mean of 16%. The results given above indicate the good preservation of RNA in freeze-substituted material and its low solubility in water. When in this case RNA content was calculated for dry mass of tissue and compared with the control, the values obtained were higher than 100% (Table II). This could be explained by a considerable loss of different substances other than RNA from the tissue. This explanation is supported by calculation of RNA content for the fresh weight of tissue in which the loss of substances other than RNA is not taken into consideration.

A considerable loss of DNA, i.e. about 17%, was found in the substituted material. After water extraction DNA loss reached 25%. The discrepancy in losses of RNA and DNA in the course of substitution is interesting in view

Table I. Dry mass and nucleic acid content in the control rat liver

	% of dry weight	Nucleic acid content expressed as mg% of P			
		in dry material		in fresh material	
		RNA	DNA	RNA	DNA
Mean	25.4	43.3	82.3	10.9	20.6
Standard deviation	1.8	11.6	26.9	2.6	5.7
Variation coefficient	7.1	26.8	32.7	23.9	27.7

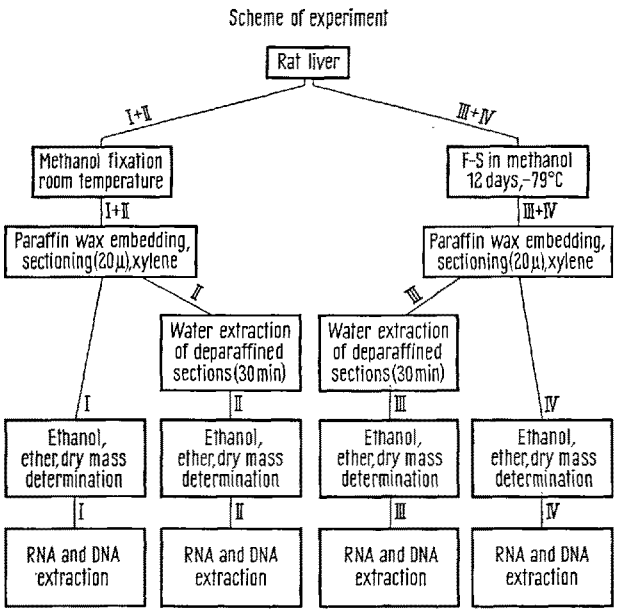

Fig. 1

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Table II. Dry mass and nucleic acid content in the rat liver processed in different ways

Experimental groups	% of dry weight	% of the nucleic acid content			
		in the dry material		calculated for the fresh weight	
		RNA	DNA	RNA	DNA
Alcohol-fixed material after water extraction	23.5 S.D. 1.6 V.C. 6.8	101.8 S.D. 17.6 V.C. 17.3	101.3 S.D. 21.9 V.C. 21.6	92.9 S.D. 16.2 V.C. 17.4	92.9 S.D. 19.5 V.C. 21.0
Freeze-substituted material	23.3 S.D. 3.0 V.C. 12.9	109.3 S.D. 15.8 V.C. 14.5	90.6 S.D. 21.4 V.C. 23.6	97.9 S.D. 16.7 V.C. 17.1	82.9 S.D. 22.1 V.C. 26.7
Freeze-substituted material after water extraction	21.6 S.D. 1.7 V.C. 7.9	125.4 S.D. 26.9 V.C. 21.5	89.2 S.D. 17.7 V.C. 19.8	107.6 S.D. 20.2 V.C. 18.8	76.4 S.D. 14.6 V.C. 19.1

of the fact that, in the material normally fixed by methanol, losses and solubility of both nucleic acids are the same (Table II). An explanation of the high losses of DNA in the course of substitution cannot be attempted on the basis of the investigation outlined in this paper. MIYAJI and PRICE[6] have stated that in a solution devoid of electrolytes the disintegration of sodium deoxypentose nucleate is speeded up by a temperature rise and slowed down by presence of electrolytes. The conditions used in our experiments favoured the disintegration of DNA molecules in this respect. It may be assumed that the disintegration is high because of the incomplete denaturation of DNA in the substituted material. It remains unclear why there is a difference in the solubility of RNA and DNA in identical experimental conditions.

Conclusions. (1) In the methanol-substituted tissue, no losses of RNA were found whereas DNA content was diminished by 17%. (2) Methanol-substituted material subsequently extracted by water loses a certain amount of tissue substance, which was expressed by a 16% loss in dry weight as compared to the control. DNA is partly eluted as well since its content in tissue is about 76%. RNA is not extracted by water. (3) Methanol substitution may be recommended as a method for RNA determination but is inadequate for quantitative determination of DNA.

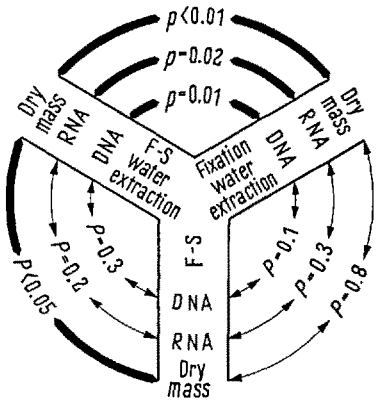

Fig. 2. Statistical significance of the differences of dry mass and nucleic acid content in the experimental groups.

Résumé. Les auteurs ont déterminé le contenu en acides nucléiques dans le foie du rat après la congélation-dessication dans du méthanol. Après cette technique, une grande partie de DNA était soluble dans l'eau.

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Department of Histology and Embryology, Medical Academy, Warsaw (Poland), November 6, 1964.

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Effect of Polyribonucleotides on Amino Acid Incorporation by Chloroplast Ribosomes

That chloroplasts can synthesize protein has been demonstrated by many workers[1–4]. Ribonucleoprotein particles have recently been isolated from chloroplasts by MIKULSKA et al.[5]. While this work was in progress, two reports[6,7] were published regarding the protein synthesis by chloroplast ribosomes. We wish to report our results on the effect of polyribonucleotides on amino acid incorporation by chloroplast polyribosomes.

Chloroplasts were isolated from spinach leaves according to the method of GORHAM[8]. 10–20 g fresh chloroplasts were disrupted with 20 vol 0.10 *M* Tris buffer pH 7.0 by constant stirring in cold for 1 h and then centrifuged at 10,000 *g* for 30 min. The pellet constitutes the grana, and the supernatant stroma thus obtained was further subjected to centrifugation at 105,000 *g* for 2 h. The pellet constitutes the RNP-particles and from the supernatant pH 5 fraction was isolated. Both the fractions were dispersed in a minimal amount of 0.10 *M* Tris buffer pH 7.0. The total volume was adjusted to 2–5 ml according to the initial amount of chloroplasts taken. Polyribonucleotides were prepared with *Azetobacter vinelandii* polynucleotide phosphorylase, according to the method of GRUNBERG-MANAGO et al.[9]. After 1 h incubation the reaction was stopped with 1 ml of 1 *M* perchloric acid. The precipitate was washed 7–8 times with 10 ml 0.2 *M* perchloric acid, after dissolving once in KOH, and reprecipitated with perchloric acid. Finally the precipitate was dispersed in a few drops of NH_4OH and the volume was made up to 1.0 ml. An aliquot of this was plated and counted. Protein was estimated by Biuret test[10], after removing chlorophyll with alcohol and acetone whenever necessary.

Ribosomal particles were isolated from chloroplasts and, once sedimented at 105,000 *g*, it was found to be difficult to get into clear solution and therefore the stroma and pH 5 fractions were subjected to analytical ultracentrifugation. Ribosomal particles of various sizes (20 S – 130 S) were found to be present in the stroma fraction as has been shown in the Figure. With pH 5 only 20 S particles were obtained. When RNP particles and pH 5

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