CXCIV. NARCOTINE AS THE ALLEGED PRECURSOR OF VITAMIN C.

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(Received August 25th, 1932.)

ACCORDING to Rygh and Rygh [1931] and Rygh, Rygh and Laland [1932], when the neutralised juice from ripe oranges is extracted with peroxide-free ether, an antiscorbutic fraction, consisting of an oily substance with some crystals, is obtained. If on the other hand the juice from unripe green oranges is used the resulting product consists almost exclusively of crystalline narcotine, which does not possess any antiscorbutic activity. They therefore consider that narcotine is present in unripe oranges as a provitamin and as the fruit ripens² it is converted into vitamin C. This view received further support from an observation made by them [Rygh and Rygh, 1931; 1932] that when methyl α -narcotine, as prepared by demethylation of narcotine, is administered to guinea-pigs on a scorbutic diet, the development of the usual scorbutic lesions is prevented, although the death of the animals supervenes within about 5 weeks. The above experiments were repeated by us but we were unable to confirm any of the results obtained by Rygh and his collaborators.

ATTEMPTED ISOLATION OF NARCOTINE FROM ORANGE JUICE.

The freshly expressed juice (2000 cc.) of 214 green, unripe oranges (Valencia Late) from South Africa was extracted with purified ether (twice). After being made slightly alkaline to litmus the juice was again extracted with purified ether (four times). These four ether extracts were united, washed with water and dried with anhydrous sodium sulphate. The residue left after removal of the ether was examined for the presence of narcotine. No fraction with the characteristic properties of narcotine could be isolated but a slight precipitate was produced with Mayer's reagent. The precipitate did not give Labat's colour reaction [Labat, 1909] nor did it give any narcotine on decomposition with hydrogen sulphide. Narcotine when precipitated with Mayer's reagent

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² There is no evidence that unripe fruits or vegetables have a lower vitamin C concentration in their juices than ripe ones.

gives this reaction and when the precipitate from as little as 0.3 mg. of narcotine is decomposed with hydrogen sulphide the presence of the alkaloid can be detected.

In view of the weakly basic properties of narcotine the above first ether extracts were also examined for the presence of this alkaloid but no narcotine could be found.

Three other varieties of orange: South African Navel (unripe), Seville (partially unripe) and Jaffa (partially unripe) were examined in the same way with similar results. In all these experiments the efficiency of the extraction process was checked by adding narcotine to a portion of the orange juice and extracting as indicated above. In each case the narcotine was readily recovered as crystals which melted at 173° and after recrystallisation at 176°.

Ott and Packendorff [1932] have reported the isolation of narcotine from lemons. In one case a yield of 600 mg. of the crude product was obtained from 600 lemons. In other experiments the yield was much less and at times no narcotine could be obtained.

ATTEMPTED PREPARATION OF ANTISCORBUTIC CONCENTRATE FROM LEMON JUICE BY RYGH, RYGH AND LALAND'S METHOD.

Lemon juice was concentrated to about one-fifth of its volume at 25° under reduced pressure and the concentrated solution extracted with peroxide-free ether in a mechanical shaker in an atmosphere of pure nitrogen until no more colouring matter was removed from the lemon juice (three extractions). It was then neutralised to litmus using 40 % NaOH at first and finally 10 % NaOH and extracted again as above three times with peroxide-free ether. The combined second ethereal extracts were dried over CaCl₂ and concentrated at 30° under reduced pressure to a small volume to which 1 % aqueous tartaric acid solution was added. The residual ether was then distilled off also under reduced pressure. As the ether was removed some solid matter separated out. We found that when the ethereal extract was taken to dryness it was difficult to dissolve the entire residue in tartaric acid. In the biological tests an evenly distributed suspension of the above preparation was therefore used. The doses were prepared daily and were administered immediately, avoiding exposure to air as much as possible. During the week-end a double dose was given. Doses of $\gamma/10$, 1 γ , 1 mg. and 2 mg. were tested, using three guinea-pigs (of about 300 g. wt.) on each dose. All the twelve animals succumbed to scurvy with the usual symptoms as if they were on the basal diet alone, showing the characteristic picture of the disease at the *post mortem* examination.

AN EXAMINATION OF METHYLNORNARCOTINE FOR ANTISCORBUTIC ACTIVITY.

We have already reported [Smith and Zilva, 1932] some negative results obtained with narcotine which had been hydrolysed with concentrated hydrochloric acid continuously for 48 hours and 72 hours respectively. Several more preparations demethylated under various conditions have since been

tested and were also found to be inactive. Demethylation of narcotine as carried out by Matthiessen and Foster [1867; 1868] and by Matthiessen [1869, 1, 2] yielded in our hands very impure methyl*n*arcotine and dimethyl*n*arcotine containing much oxidised material. When demethylation was carried out in an atmosphere of nitrogen the rate of the reaction was found to vary in accordance with the purity of the nitrogen; the presence of even traces of oxygen increases the velocity of the reaction. This will be seen from Table I which sets out the degree of hydrolysis obtained by heating 5 g. of narcotine with 50 cc. of concentrated hydrochloric acid at 100°.

Table I.

Conditions							% MeO in crude product	Time of hydrolysis, hours
Aerobic...	...	...	...	...	...	...	2.1	89
In commercial nitrogen	...	...	...	...	...	...	2.9	"
Solution covered with half an inch of paraffin wax							3.6	"
In purified nitrogen	...	...	...	...	...	...	6.4	"
In highly purified nitrogen	...	...	...	...	...	...	10.2	"

Methyl*n*arcotine hydrochloride contains MeO, 7.4 %.

Dimethyl*n*arcotine hydrochloride contains MeO, 14.2 %.

In this communication we are describing only two typical experiments, one dealing with methyl*n*arcotine prepared under strictly anaerobic conditions and another in which less strict anaerobic conditions were observed.

Exp. 1. 10 g. of narcotine in 100 cc. of freshly distilled oxygen-free hydrochloric acid were heated at 100° for 126 hours continuously under strict anaerobic conditions, using carbon dioxide as inert gas. The colourless reaction mixture became coloured rose-pink after being heated for 48 hours; after 72 hours a little methyl*n*arcotine was deposited and increased in quantity as the reaction proceeded. On completion, the hydrochloric acid was distilled off *in vacuo* and the rose-pink amorphous residue was dissolved in 188 cc. of oxygen-free water, forming a light red-wine coloured solution (solid content, 0.0544 g. per cc.). All operations were carried out under strict anaerobic conditions.

Part of the solution was transferred to 1 cc. ampoules in an atmosphere of carbon dioxide and sealed without the solution coming into contact with air. The ampoules were reserved for biological tests, which are described below, and the remaining solution was used for the chemical examination of the product.

Chemical examination. The remainder of the solution was treated with sufficient concentrated hydrochloric acid to produce maximum precipitation. The white amorphous precipitate (6.0 g.) was filtered off as quickly as possible and dried over phosphorus pentoxide in a high vacuum. The precipitate developed a slight grey-lavender colour on drying. Found, after drying in a vacuum at 95° for half an hour and cooling in an atmosphere of dry nitrogen; C, 56.7; H, 5.2; N, 3.2; Cl, 8.5; MeO, 7.5 %. Calculated for $C_{20}H_{19}O_7N$, HCl;

10 γ

20 γ

30 γ

100 γ

1.4 mg.

2.7 mg.

Negative
control

Positive
control

C, 56.9; H, 4.8; N, 3.3; Cl, 8.4; MeO, 7.4 %. The mother-liquor was water-clear and contained nothing except a trace of methyl*nornarcotine*.

Fractionation of the remaining methyl*nornarcotine* (5.6 g.) gave 0.5 g. of less soluble material (MeO, 6.5 %) which consisted of methyl*nornarcotine* hydrochloride, *nornarcotine* hydrochloride and oxidised material; 0.3 g. of more soluble material (MeO, 8.8 %) which consisted of a mixture of methyl*nornarcotine* hydrochloride and dimethyl*nornarcotine* hydrochloride; and 4.1 g. of methyl*nornarcotine* hydrochloride (MeO, 7.4 %). The latter still contained a trace of oxidised material which was removed by prolonged treatment with acetone in the cold.

Methyl*nornarcotine* hydrochloride is a colourless, amorphous powder, M.P. 253° (corr. dec.), $[\alpha]_{\text{H}_2\text{O}}^{22}$ yellow + 333°, $[\alpha]_{\text{D}}^{22} + 382^\circ$ (c, 1.2 in water). It is readily soluble in water, almost insoluble in dilute hydrochloric acid, sparingly soluble in concentrated hydrochloric acid. It does not crystallise as stated by Rygh but separates from solvents in small white granules. The base is conveniently prepared by dissolving the hydrochloride in pyridine and pouring the solution into water. Methyl*nornarcotine* is a cream-coloured amorphous powder, melting point 227° (corr. dec.). It is sparingly soluble in most organic solvents but dissolves readily in pyridine. Attempts to prepare crystalline salts gave amorphous products which showed no tendency to crystallise. The esters prepared by acetylation or benzylation were also amorphous. The base tends to oxidise on drying for analysis; found C, 61.7; H, 5.1; N, 3.6; MeO, 7.9 %. $\text{C}_{20}\text{H}_{19}\text{O}_7\text{N}$ requires C, 62.3; H, 5.0; N, 3.6; MeO, 8.0 %.

Biological test. The following doses were tested, 10γ, 20γ, 30γ, 100γ, 1.4 mg. and 2.7 mg., using 6 guinea-pigs (of about 300 g. wt.) on each dose. A layer of liquid paraffin was introduced immediately after opening the ampoules in order to protect the contents from the air. A quantity of the solution equivalent to the dose was then quickly removed by means of a graduated capillary pipette and diluted with oxygen-free, distilled water. The time which elapsed between the removal of the dose from the ampoule and the end of the delivery of the dose was under a minute. All the animals developed scurvy in the usual time and died of the disease after about 30–35 days. The *post mortem* examinations revealed typical scurvy. X-ray photographs were taken of the ribs of all the animals in this experiment but no differences between the animals which received methyl*nornarcotine* and those which were on a basal diet alone, such as were observed by Rygh and Rygh [1931], could be discerned. This will be seen from Plate VI in which X-ray photographs of the ribs of one animal in each group are given. We should like to take this opportunity of expressing our indebtedness to Miss H. M. Bruce of the National Institute for Medical Research, Hampstead, for kindly preparing the radiographs.

Exp. 2. Demethylation was carried out under less strict anaerobic conditions. 10 g. of narcotine in 100 cc. of concentrated hydrochloric acid were heated at 100° for 50 hours continuously in an atmosphere of commercial

nitrogen. The hydrochloric acid was then distilled off and the residue (10.2 g.) dissolved in oxygen-free water (112 cc.). Part of the solution (50 cc.) was sealed up in 1 cc. glass ampoules under commercial nitrogen for biological tests. The crude methyl α -narcotine (MeO, 8.7 %) was found to consist of about 80 % methyl α -narcotine with 20 % of dimethyl α -narcotine and a trace of oxidised material.

The tests in this experiment were carried out under the same conditions as in Exp. 1 except that four animals only were used on each dose. As in Exp. 1 no deviation in the behaviour of the experimental animals from that of those on a scorbutic diet alone could be observed.

Since the completion of this investigation the results of Dalmer and Moll's [1932] repetition of Rygh's work have come to our notice. Like ourselves they were unable to isolate narcotine from oranges or to prepare active concentrates from lemons by Rygh, Rygh and Laland's method, nor could they detect any antiscorbutic activity in methyl α -narcotine. Tillmans and Hirsch [1932] and Harris *et al.* [1932] also could not detect any antiscorbutic activity in methyl α -narcotine.

SUMMARY.

Narcotine could not be detected in unripe or partially ripe oranges.

Attempts to prepare an antiscorbutic concentrate from lemon juice according to Rygh, Rygh and Laland's method failed.

The administration of a number of preparations of methyl α -narcotine, obtained by demethylation of narcotine under various conditions, to guinea-pigs on a scorbutic diet failed to modify the development or character of the ensuing scorbutic symptoms. The experimental animals died in the usual time of typical scurvy.

Our thanks are due to Mr A. Bennett and Mr H. C. Clarke for carrying out the analyses in this investigation.

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