Cardiazol administré 30 min après l'amide (II). A la dose de 20 mg/k en i.v., elle s'oppose à l'excitation motrice générale provoquée chez le chien chloralosé par l'injection intraveineuse de 5 mg/k de Cardiazol.

Contrairement au composé V, les substances II et III sont dépourvues de propriétés antipyrétiques et anti-inflammatoires. La propynoxybenzamide (II) montre un effet préventif et correctif vis-à-vis tremblements provoqués chez la souris par la trémorine.

En conclusion, du fait qu'elle est dépourvue d'action hypnotique, la propynoxybenzamide (II) semble être un sédatif moteur pur. Cependant son application thérapeutique n'est pas à envisager en raison des effets toxiques qu'elle exerce sur le parenchyme hépatique[8,9].

*Summary.* Description of the synthesis and pharmacological properties of 2-propynoxybenzamide. This substance has a depressive effect on the motor activity without any hypnotic action. However, its toxic effect on the liver parenchyma prevents its use in therapy.

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*Services de Recherche LABAZ, Division Pharmaceutique de la SBA-PCM, Bruxelles (Belgique), le 24 avril 1962.*

[8] M. A. GEREBTZOFF, communication personnelle.

[9] Nous remercions M. URBAIN, J. ROSSENEU et A. HOSSLET pour l'aide technique qu'ils nous ont apportée.

## Hydrolysis of Riboflavin by Plants

The riboflavin-containing nucleotides, flavin adenine dinucleotide and flavin mononucleotide are found in many plants, and pathways have been described for the biosynthesis of these nucleotides[1-3]. Alternate pathways of riboflavin metabolism may also exist in plants. Thus, SCHOPFER[4] has shown that lumichrome, as well as riboflavin, accumulate in the cell vacuoles of the upper epidermis of the bulb scales of *allium*. There have been no reports of the enzymatic breakdown of riboflavin in plants, although YANAGITA and FOSTER[5] have shown that *Pseudomonas riboflavina*, isolated from soil by enrichment culture methods, hydrolyzes riboflavin to lumichrome (6,7 dimethylalloxazine). Similarly, MILES and STADTMAN[6] and FALL and PETERING[7] have demonstrated the breakdown of riboflavin to 6,7-dimethyl-(2'hydroxyethyl) isoalloxazine by bacteria grown on media containing riboflavin as the major carbon source. The present communication describes an enzyme system from *Crinum longifolium* which converts riboflavin quantitatively to lumichrome.

20 g of thoroughly cleaned and sliced hypogeous portion of the plant *Crinum longifolium* were homogenized in 60 ml of Tris (0.01 *M*)-versene (0.01 *M*)-sucrose (0.25 *M*) buffer, pH 7.2. The homogenate was allowed to stand for 4 h at 0–5° and was then strained through cheesecloth and dialysed against 2 l of water for 8 h. The precipitate obtained by centrifuging the dialysed extract at $1500 \times g$ was discarded and the supernatant solution used as the enzyme.

The identity of the reaction product, lumichrome, was established by the following experiment: a reaction mixture containing 5.0 ml of sodium phosphate buffer, pH 7.2, 0.2 *M*; 5.0 ml of riboflavin, 0.02 m*M*; 1.0 ml of reduced glutathione, 0.2 m*M*; and 9.0 ml of the enzyme preparation was incubated at 37° for 1 h. Controls without enzyme and with the enzyme heated at 100° for 10 min were incubated similarly. Precaution was taken to exclude exposure to light during the experiment. The reaction was stopped by the addition of 5.0 ml of trichloracetic acid (40% w/v). The reaction mixture and the controls were centrifuged and the supernatant fluids were concentrated under reduced pressure at 40° and subjected to preparative circular paper chromatography using *n*-butanol:acetic acid:water (4:1:5, v/v) as the solvent system[8]. When examined under an ultraviolet lamp with Wood's filter (Phillips HPW, 125 W, Typ 57202 E/70), chromatograms of the reaction mixture showed a single band (Rf = 0.7) with a sky-blue fluorescence. The chromatograms of the controls did not exhibit this band. The absorption spectrum of the Rf 0.7 material, when eluted from the paper with water, had absorption maxima at 255, 346, and 398 m$\mu$ in 0.1 *N* sodium carbonate. An aliquot of this material was co-chromatographed with a sample of lumichrome prepared according to KARRER[9], in three solvent systems, *viz.*, butanol:acetic acid:water (4:1:5, v/v), butanol:propanol:water (4:4:2), and 50% methanol. In all these solvent systems the unknown material moved as a single band and could not be separated from the authentic sample of lumichrome. The other reaction product, ribitol, was identified by chromatographing the original reaction mixture in 50% methanol and spraying the chromatograms with either alkaline potassium permanganate[10] or boric acid-bromocresol purple[11]. The Rf value of authentic ribitol is 0.70 in this solvent system. The control tubes contained no free ribitol.

To establish the stoichiometry of the reaction, assay mixtures containing 0.4 ml of phosphate buffer, pH 7.2, 0.1 *M*; 0.1 ml of reduced glutathione, 0.2 m*M*; 0.5 ml of riboflavin, 0.02 m*M*; and 1.0 ml of the enzyme, total volume 2.0 ml, were incubated at 37° for 0, 30, 60, 90, and 120 min, respectively. The reaction was stopped at various time intervals by the addition of 0.5 ml of trichloroacetic acid (40% w/v) and the supernatant liquid was subjected to circular paper chromatography, using butanol:acetic acid:water (4:1:5, v/v) as solvent system. Riboflavin (Rf = 0.45) and lumichrome (Rf = 0.7) were located in UV-light, eluted with water, and the concentration of each flavin was determined from the absorbancy measurements at 265 m$\mu$ ($\log \varepsilon = 4.89$) for riboflavin and 255 m$\mu$

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($\log \varepsilon = 4.6$) for lumichrome. From the results in Table I, it is evident that the breakdown of riboflavin to lumichrome is quantitative in this system.

Tab. I. Stoichiometry of the hydrolysis of riboflavin

| Time min | Riboflavin disappearance mμ moles | Lumichrome formation mμ moles |
|---|---|---|
| 30 | − 39.2 | + 37.6 |
| 60 | − 55.3 | + 55.1 |
| 90 | − 58.3 | + 58.1 |
| 120 | − 60.3 | + 60.1 |

Reaction mixtures contained 0.4 ml of sodium phosphate buffer, pH 7.2; 0.1 ml of reduced glutathione, 0.2 m$M$; 0.5 ml of riboflavin, 0.02 m$M$; and 1.0 ml of the enzyme in a total volume of 2.0 ml. The reaction mixtures were incubated at 37° for the time intervals specified in Table I.

Tab. II. Distribution of the riboflavin hydrolyzing enzyme in plants

| Plant[a] | Natural order | Activity[b] per mg protein |
|---|---|---|
| *Crinum longifolium* | Amaryllidaceae | 18.3 |
| *Crinum asiatica* | Amaryllidaceae | 16.5 |
| *Crinum amabale* | Amaryllidaceae | 16.5 |
| *Haemanthus multiflorus* | Amaryllidaceae | 17.2 |
| *Polyanthus* Sp. | Amaryllidaceae | 13.5 |
| *Amaryllis* Sp. | Amaryllidaceae | 12.8 |
| *Allium cepa* | Lilliaceae | 6.3 |
| *Canna indica* | Cannaceae | 6.9 |

[a] Hypogeous part processed as described in text.
[b] Activity is expressed as mμ moles of lumichrome synthesized at 37° in 60 min.

A number of plants were screened for the presence of the riboflavin-lumichrome conversion. Although this enzyme does not occur generally in plants, it is found in members of the natural orders *Amarylliaceace*, *Lilliaceace*, and *Cannaceace*. Table II gives the activity per mg protein in extracts of these plants. It is perhaps significant that the enzyme system catalyzing the hydrolysis of riboflavin occurs in monocotyledonous plants, in view of the reported hormonal activity of lumichrome[12]. The optimum conditions for activity in all cases were pH 7.2 and 37°; catalytic amounts of reduced glutathione were also required.

Our observation that certain plant extracts can catalyse the hydrolysis of riboflavin to lumichrome suggests that there are alternate pathways of riboflavin metabolism in plant kingdom[13].

*Résumé.* Il a été prouvé que l'hydrolyse de la riboflavine à l'aide d'une enzyme se produit dans les extraits de plantes appartenant aux familles des *Liliacées*, *Amaryllidacées* et *Cannas* (ou Balisier).

On a trouvé le lumichrome et le ribitole parmi les produits de l'hydrolyse. La réaction est stœchiométrique, les conditions favorisant une activité optimale sont les suivantes: pH 7.4, température 37°, quantités catalytiques de glutathione reduit.

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## Asymmetry and Yield in *Cocos nucifera* L.

The leaves of *Cocos nucifera* L. are arranged in a right-handed of left-handed spiral, the angle between corresponding leaves in successive whorls being about 30°. The frequency of lefts among 3028 trees in India was 52.05% (Davis[1]) and among 13842 trees elsewhere it was 52.90%. The asymmetry is not inherited (Davis[1]) and has been regarded as trivial.

Of the 384 trees used at the Central Coconut Research Station, Kayangulam, Kerala (India), for trials of micronutrients, 177 were left-spiralled. They were divided into three groups, healthy, moderately affected by a major Root (wilt) disease, and severely affected. The mean number of nuts per year borne by the right-spiralled and left-spiralled trees in these groups between 1955 and 1960 inclusive are shown in the Table.

The figures for the healthy trees give $t = 2.721$ (126 degrees of freedom). The probability for a difference of that magnitude or more being small ($P = 0.0076$), the assumption that left-spiralled trees give higher yields is strongly substantiated. The figures for the diseased trees, though not quite significantly different, strongly reinforce the significance of those for the healthy trees.

As neither the nuts nor the kernels from the two types of trees were weighed separately, it is, of course, possible that the total mean weight of copra produced by the left-spiralled trees was not greater than that from the right-spiralled. Nor is it claimed that all races of coconut, in all soils and climates, behave in this way. However, the biological fact here presented is, I believe, novel. Many explanations can be suggested for it, of which I hope to discuss some elsewhere[2].

Average number of nuts produced per tree per year

| Condition of trees | No. of trees right | No. of trees left | Nuts per tree/year right | Nuts per tree/year left |
|---|---|---|---|---|
| Healthy | 70 | 58 | 53.93 | 65.25 |
| Moderate disease | 67 | 61 | 32.60 | 35.98 |
| Severe disease | 70 | 58 | 18.58 | 23.15 |

[1] T. A. Davis, J. Genet., *58*, 12 (1962).
[2] Help received from the Indian Central Coconut Committee for my service is gratefully acknowledged.