Tab. II. Fatty acids in the freeze-dried homogenate of epiphyseal cartilage. Determination carried out by gas-liquid chromatography

Fatty acids		Percentage referred to total fatty acids
Saturated		43.00
Unsaturated (mono-)		47.50
Unsaturated (poly-)		9.50
Lauric	C_{12}	0.20
Myristic	C_{14}	2.30
Palmitic	C_{16}	24.50
Palmitoleic	$C_{16}(-2H)$	9.50
Stearic	C_{18}	15.00
Oleic	$C_{18}(-2H)$	37.10
Linoleic	$C_{18}(-4H)$	4.10
Eicosotrienoic	$C_{20}(-6H)$	0.90
Arachidonic	$C_{20}(-8H)$	3.20
Other fatty acids (traces)		3.20

investigations in order to correlate, if possible, our findings to the mineralization process of the tissue.

Riassunto. Nella cartilagine metafisaria di maialini neonati omogenata e liofilizzata sono stati determinati, con metodi fisici e chimici, i lipidi totali, il fosforo totale, il fosforo lipidico, il colesterolo totale, il colesterolo libero, l'azoto totale e gli acidi grassi saturi, insaturi e polinsaturi. La determinazione degli acidi grassi è stata effettuata mediante la gas-cromatografia.

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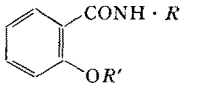
Propynoxy-2 benzamides et analogues[1]

L'allyloxy-2 benzamide possède des propriétés analgésiques[2] et narcotiques[3] plus intenses que celles de la salicylamide. Ayant établi au cours des travaux dans la série des carbinols et carbamates propynyliques les avantages que présente le radical propargyle sur le radical allyle[4], nous avons voulu nous rendre compte si cette différence se retrouverait dans les éthers correspondants de la salicylamide.

Tab. I. Constantes physiques



No.	R	R'	F°C	Analyses N tr.%	N calc.%
I	-H	$-CH_2CH=CH_2$	97 ± 1[a]		
II	-H	$-CH_2C\equiv CH$	151 ± 1	7,99	8,02
III	$-COCH_3$	$-CH_2C\equiv CH$	108 ± 1	6,44	6,29
IV	$-COCH_3$	$-CH_2CH=CH_2$	61 ± 1	6,38	6,34
V[b]	$-COCH_3$	-H	149 ± 1		

[a] Voir [5], F. 97–98°. [b] Labazyl®-Labaz.

Dans ce but, nous avons préparé la propynoxy-2 benzamide (II) et quelques analogues (Tableau I). Ces substances ont été obtenues par réaction des bromures d'allyle ou de propargyle ou du tosylate de propargyle avec la salicylamide ou la N-acétyl salicylamide en présence de carbonate de potassium dans l'acétone. Les produits se présentent sous forme cristalline incolore.

Testée au bâton tournant[6] la propynoxybenzamide (II) est un sédatif moteur; elle est moins active que l'allyloxybenzamide (I), mais sa durée d'action est beaucoup plus longue. Les dérivés III et IV sont nettement moins actifs.

Le composé II ne possède pas de propriétés hypnotiques, même si l'on injecte des doses toxiques. Par contre,

Tab. II. Activité sédative au bâton tournant, LD_{50}, index thérapeutique et effet analgésique

N°	ED_{50} sédative mg/k, i.p. (souris)	LD_{50} mg/k, i.p. (souris)	Index thérapeutique	Activité analgésique (V = 1)
I	80	340	4,25	4–5
II	125	325	2,60	2
III	175			2
IV	170	-		2
V	-	-		1

l'allyloxybenzamide (I) montre des effets hypnotiques à la dose de 250 mg/k.

Les substances I et II potentialisent au même degré la narcose barbiturique lorsqu'elles sont injectées 30 min avant le barbiturique (Hexobarbital ou Pentothal) et à une dose non sédative par elle-même (50 mg/k i.p.). L'activité analgésique de la substance I, déterminée au rectodolorimètre[7] et exprimée par rapport au dérivé V, dépasse celle des substances II, III et IV.

La propynoxybenzamide (II) potentialise l'effet analgésique de la péthidine examiné par la méthode de Haffner et la méthode du rectodolorimètre. A la dose de 150 mg/k en i.p., la substance II retarde chez la souris les effets convulsivants puis mortels d'une dose i.p. de 100 mg/k de

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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.

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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 mμ 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 mμ ($\log \varepsilon = 4.89$) for riboflavin and 255 mμ

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