Brèves communications - Kurze Mitteilungen
Brevi comunicazioni - Brief Reports

Microbiological Hydroxylation of Certain Indole Alkaloids

While microbiological transformations have been widely studied in the steroid field, there are only few examples of such transformations of alkaloids.

When apoyohimbine (Ia)[1], β-yohimbine methyl ether (IIa)[2], and 3-epi-apoyohimbine (IIIa)[3] were aerobically incubated (200 γ/ml) in deep culture fermenters with a 48 h growth of *Cunninghamella Blakesleana* Lendner on a pea meal medium for 24–72 h they were transformed into more polar compounds. Details of the fermentations (followed by means of paper chromatography in the B5 system of BUSH[5]) and the isolation of the fermentation products will be presented in a forthcoming paper.

Paper chromatography of the apoyohimbine fermentations revealed two transformation products of $R_F = 0{\cdot}60$ (green fluorescence after irradiation with U.V.-light) and $R_F = 0{\cdot}38$ (purple spot after spraying with a butanolic solution of diazotized sulfanilamide followed by alkali[6]) respectively. The main transformation products of IIa and IIIa were also detected with this phenol reagent ($R_F = 0{\cdot}18$ and $0{\cdot}34$ respectively).

The structures Ib, IIb, and IIIb were assigned to the 'phenolic' transformation products of Ia, IIa, and IIIa for the following reasons: The microanalysis in connection with their ready solubility in aqueous alkali, their ability to couple with diazonium salts, and the finding of two active hydrogen atoms (ZEREWITINOFF) indicated the presence of one phenolic hydroxyl group. A 17-enol in I and III could be excluded since the phenolic properties were retained after catalytical hydrogenation of the 16, 17-double bond. Because of the striking resemblance of the ultraviolet spectra of these compounds [λ_{max} 274 mμ, log ε 3·97; 295–300 mμ (shoulder), log ε 3·65; λ_{min} 248 mμ, log ε 3·61 (0·01n methanolic HCl)] to that of serotonin the the hydroxyl group could be located to the 10-position[7].

[1] G. BARGER and E. FIELD, J. chem. Soc. *123*, 1038 (1923)

[2] W. O. GODTFREDSEN and S. VANGEDAL, Acta chem. Scand. *11*, 1013 (1957).

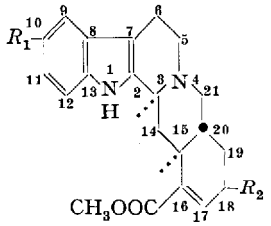
[3] The new compound IIIa was prepared by dehydration of pseudoyohimbine[4] with phosphorus oxychloride in pyridine. M. p. 91–93; $[\alpha]_D^{20} = +85°$ (pyridine).

[4] W. O. GODTFREDSEN und S. VANGEDAL, Acta chem. Scand. *10*, 1414 (1956).

[5] W. O. GODTFREDSEN and S. VANGEDAL, Acta chem. Scand. *11*, 1013 (1957); *10*, 1414 (1956). I. E. BUSH, Biochem. J. *50*, 370 (1952).

[6] For details, see R. J. BLOCK, E. L. DURRUM, and G. ZWEIG, *Paper Chromatography* (Academic Press, N.Y. 1955), p. 95.

[7] A similar hydroxylation is the recently reported conversion of tryptophan with *Chromobacterium violaceum:* C. MITOMA, H. WEISSBACH, and S. UDENFRIEND, Arch. Biochem. Biophys. *63*, 122 (1956).

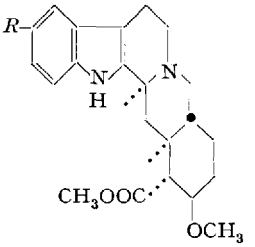


I

a) $R_1 = R_2 = H$

b) $R_1 = OH$, $R_2 = H$ — $C_{21}H_{24}N_2O_3$; m.p. 259–261°C; $[\alpha]_D^{23} = +34{\cdot}5 \pm 1°$ (pyridine)

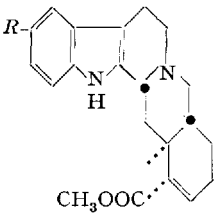
c) $R_1 = H$, $R_2 = OH$ — $C_{21}H_{24}N_2O_3$, HCl; m.p. 273–274°C; $[\alpha]_D^{23} = +64° \pm 1°$ (methanol)



II

a) $R = H$

b) $R = OH$; $C_{22}H_{28}N_2O_4$; m.p. 289–290°C; $[\alpha]_D^{23} = -14 \pm 3°$ (pyridine)



III

a) $R = H$

b) $R = OH$; $C_{21}H_{24}N_2O_3$, $^1/_2\,C_6H_6$; m.p. 146–148°C; $[\alpha]_D^{23} = +40 \pm 1°$ (pyridine)

With vanillin in concentrated hydrochloric acid Ib, IIb, and IIIb gave a very characteristic cornflower blue colour in contrast to the red colour obtained with other alkaloids of the yohimbine type.

The second fermentation product from apoyohimbine was monohydroxylated but not phenolic; its ultraviolet spectrum was practically identical with that of Ia. Although an unambiguous proof of the structure of this compound is still lacking, there is some evidence for the location of the hydroxyl group to the 18-position (Ic): Treatment in chloroform with active manganese dioxide yielded an amorphous product which showed strong infrared absorption bands at 1601, 1680, and 1723 cm^{-1} corresponding to the 16,17-double bond, the keto group and the ester carbonyl in the grouping CH_3OOC-  $-O$.

W. O. Godtfredsen, G. Korsby, H. Lorck, and S. Vangedal

Research Department, Leo Pharmaceutical Products Copenhagen, December 3, 1957.

Zusammenfassung

Aerobe Kulturen von *Cunninghamella Blakesleana* Lendner hydroxylieren Apoyohimbin, β-Yohimbinmethyläther und 3-Epiapoyohimbin in der 10-Stellung. Aus Apoyohimbin wurde auch ein zweites Umwandlungsprodukt erhalten, vermutlich 18-Hydroxyapoyohimbin.

A Series of New Compounds Inhibiting the Acetylation of Choline *in vitro*

Introduction.—In previous work, some derivatives of phenylacetic acid were shown to inhibit the enzymatic acetylation of both sulfanilamide[1] and choline[2].

In this paper, we deal with the experiments performed with other acyl-derivatives on the choline acetylating system, in order to establish some relationship between chemical structure and activity, and to investigate the mechanism of the inhibition.

We advanced the hypothesis, as suggested by Cottet for phenylethylacetic acid[3], that these acyl-derivatives might inhibit the synthesis of acetylsulfanilamide and acetylcholine by preventing the acetylation of coenzyme A, the first step, common to both systems, in the acetylating reactions. It was likely that, using chemically preformed acetyl-coenzyme A, the inhibiting activity would disappear.

Experimental.—Choline acetylase was prepared from acetone-dried powder of rabbit brain and partially purified by fractionated ammonium sulfate precipitation, according to Nachmansohn's method[4].

Coenzyme A was supplied from Pabst Laboratories.

Acetyl-Coenzyme A was prepared by acetylating CoA with acetic anhydride. After the acetylation, the preparation was lyophilized to remove acetic anhydride and then dissolved in phosphate buffer at pH 7·2. Chromatographic controls were carried out on an aliquot of the preparation[5].

Inhibiting compounds marked Th have been kindly supplied by Cottet (Theraplix, Paris); compounds marked M.G. were synthetized by Cavallini and Massarani (Laboratori Maggioni, Milano)[6]; compounds marked L were synthetized by Carani (Laboratori Lofarma, Milano).

Acetylcholine assay was performed on the contraction of frog rectus muscle[7]. Similar results were obtained with chemical determinations[8], but we used bioassay as a routine test, since some compounds were found to interfere in the colorimetric reaction. None of the compounds tested, at the doses employed, interfered with the biological assay.

Results

(a) *Inhibition in acetylcholine synthesis with various compounds.* In Table II the inhibiting values obtained with the various substances are presented.

(b) *Experiments with different doses of CoA or choline acetylase.* Some experiments were performed by varying the different components of the enzymatic system.

Increased amounts of choline, acetate or ATP do not affect the inhibiting activity of the tested compounds.

On the contrary, a decrease in the percentage inhibition was obtained by increasing coenzyme A or choline acetylase in the system. Some results are summarized in Table I. The most striking effects were obtained by contemporary increase of CoA and choline acetylase; even when larger amounts of acetylcholine were synthetized, the inhibition disappeared.

Table I

Inhibitor and doses	Enzymatic solution (choline acetylase) in ml	% inhibition	
		in presence of 10 γ of CoA	in presence of 100 γ of CoA
—	0·25	(110) * —	(150) * —
L 10 1 μmole	0·25	77	49
MG 1763 1 μmole	0·25	34	4
—	0·50	(170) —	(212) —
L 10 1 μmole	0·50	49	0
MG 1763 1 μmole	0·50	15	0
—	1	(162) —	(216) —
L 10 1 μmole	1	22	0
MG 1763 1 μmole	1	9	0

* The γ of synthetized acetylcholine are reported in brackets.

(c) *Experiments with acetyl-coenzyme A.*—In Table III are reported the results obtained when chemically preformed acetyl-coenzyme A was substituted to coenzyme A, acetate and ATP. Even at a dose of inhibitor

[1] S. Garattini, C. Morpurgo, and N. Passerini, G. ital. Chemioterapia *2*, 60 (1955); Boll. Soc. ital. Biol. sper. *31*, 1653 (1955).

[2] S. Garattini, C. Morpurgo, B. Murelli, R. Paoletti, and N. Passerini, Arch. int. Pharmacodyn. *109*, 400 (1957).

[3] J. Cottet, A. Mathivat, and J. Redel, Pr. méd. *62*, 939 (1954).

[4] D. Nachmansohn and I. B. Wilson, Advanc. Enzymol. *12*, 259 (1951).

[5] G. D. Novelli, *Methods of Biochemical Analysis*, Interscience Publ. New York *2*, 208 (1955).

[6] G. Cavallini and E. Massarani, Il Farmaco, Ed. scient. *11*, 167 (1956). – G. Cavallini, E. Massarani, D. Nardi, and R. D. Ambrosio, J. Amer. chem. Soc. *79*, 3514 (1957).

[7] H. C. Chang and J. H. Gaddum, J. Physiol. *79*, 225 (1933).

[8] S. Hestrin, J. biol. Chem. *180*, 249 (1949).