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

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

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

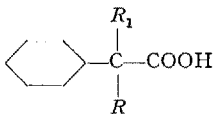
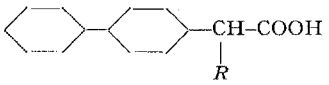
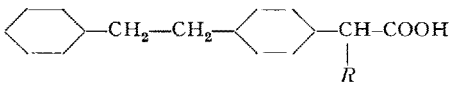
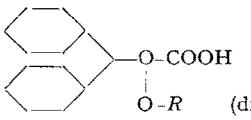
[5] G. D. Novelli, *Methods of Biochemical Analysis*, Interscience Publ. New York *2*, 208 (1955).

[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).

Table II

Code number	Formula	% inhibition of acetylcholine synthesis with doses of 0·5 μ moles	1 μ moles	2 μ moles	5 μ moles
					
	R = H R_1 = H (phenylacetic acid)	—	—	0	8
	R = ethyl R_1 = H	—	—	7	17
Th 4141	R = ethyl R_1 = ethyl	—	—	22	27
Th 4142	R = heptyl R_1 = H	27	46	67	100
	R = phenyl R_1 = H (diphenylacetic acid)	20	27	50	74
					
MG 1271	R = H (diphenylylacetic acid)	20	22	34	80
MG 1753	R = methyl	19	24	54	85
MG 1559	R = ethyl	20	33	52	88
MG 1681	R = propyl	—	39	65	—
MG 1763	R = butyl	25	52	78	100
MG 1765	R = amyl	31	64	90	100
	 (diphenylethanacetic derivatives)				
MG 1713	R = ethyl	20	34	60	90
MG 1777	R = propyl	18	46	78	100
	 (diphenylglycolic derivatives)				
L. 1	R = methyl	—	—	28	48
L. 3	R = propyl	—	—	45	68
L. 4	R = isopropyl	—	—	42	62
L. 10	R = heptyl	51	82	100	—
L. 13	R = propylphenyl	33	75	—	—
L. 14	R = ethyloxyphenyl	55	80	92	100

The enzymatic system was set up as follows: choline chloride 10 μmoles; sodium acetate 10 μmoles; ATP 6 μmoles; cysteine HCl 30 μmoles; KCl 40 μmoles; $MgCl_2$ 0·7 μmoles; $CaCl_2$ 2 μmoles; Na_2HPO_4 1·5 μmoles; DFP 0·1% one drop; choline acetylase 0·25 ml; CoA 10 γ; H_2O to 1 ml; pH 7–7·2. 3 h incubation at 37°C (90–110 γ of acetylcholine were synthesized in the absence of inhibitors).

which inhibits completely choline acetylation also in presence of large amounts of coenzyme A, there is no significant inhibition.

Table III

Inhibitor dose	CoA	AcCoA	Acetylcholine synthesized in γ	% inhibition
—	2 μmoles	–	140	–
L 10 10 μmoles	2 μmoles	–	0	100
—	–	2 μmoles	70	–
L 10 10 μmoles	–	2 μmoles	60	14
—	–	4 μmoles	110	–
L 10 10 μmoles	–	4 μmoles	110	0

The system with AcCoA contained choline 10 μmoles; DFP 0·1% one drop. Choline acetylase 1 ml in both systems.

Discussion.—Many acyl-aromatic acid derivatives have been shown to inhibit acetylcholine synthesis in CoA catalyzed systems with choline acetylase from rabbit brain.

Some speculations about the relationship between chemical structure and inhibiting activity are possible: phenylacetic acid, the first compound studied[9] has only a weak inhibiting activity. The introduction of another aromatic nucleus strongly increases the activity (see diphenylacetic, diphenylglycolic, diphenylylacetic and diphenylethanacetic acids). In every series, the introduction of a side chain steadily increases the activity and such increase is related to the length of the side chain.

As to the inhibiting mechanism, the fact that no inhibition appears when acetyl-coenzyme A is used or

[9] S. K. KOREY, D. NACHMANSOHN, and B. BRAGANZA, J. biol. Chem. *189*, 705 (1951).

when the amounts of coenzyme A and choline acetylase (therefore CoA acetylating system) are increased, suggests that these compounds do not affect the enzymatic transfer of acetic group from acetyl-coenzyme A to choline, but they inhibit the acetylation of coenzyme A. This, moreover, was likely, because our substances inhibit sulfanilamide acetylation also in pigeon liver extracts [2, 10].

Acknowledgement. We wish to thank Dr. E. MUSSINI for his cooperation in carrying out chromatographic controls on acetyl-coenzyme A.

S. GARATTINI, C. MORPURGO, and N. PASSERINI

Institute of Pharmacology, University of Milan (Italy), November 14, 1957.

Riassunto

Numerosi derivati di acidi acil-aromatici si dimostrano attivi nell'inibire la acetilazione della colina catalizzata dal Coenzima A.

Si ritiene che questa inibizione si realizzi attraverso una inibizione della acetilazione del Coenzima A.

[10] S. GARATTINI, C. MORPURGO, and N. PASSERINI (in press).

Serological Properties of Poly-L-Tyrosine Derivatives[1]

SELA *et al.*[2] have reported that guinea pigs are sensitized by the injection of polytyrosyl-gelatin, but not by gelatin alone, nor by a copolymer of tyrosine and aspartic acid. Since gelatin has been found to be antigenic in some species[3], it is not clear whether the antigenicity of polytyrosyl-gelatin is due to its gelatin moiety or to its polytyrosyl residue. We have investigated, therefore, the serological behaviour of two polytyrosine derivatives. One of them, poly-L-tyrosine-azophenylarsonate (PTA) was prepared by coupling polytyrosine ($n = 45$)[4] with an excess of diazotized arsanilic acid. The other, poly-L-tyrosyl-gelatin-azophenylarsonate (PTGA) was obtained in the same manner from poly-L-tyrosyl-gelatin[2]. Rabbits were injected subcutaneously with four 30 mg doses of PTA or PTGA directly or after addition of alum and neutralization with alkali. The first three injections were given in intervals of 3 days, the last injection after a further week. One week later the animals were bled and their sera tested with PTA, PTGA and also with arsanil-azo-bovine-γ-globulin (AsBGG) prepared from one gram of bovine γ-globulin (Armour) with 0·1 g of diazotized arsanilic acid. Neither PTA nor PTGA gave any precipitates. However, the serum from a rabbit injected with alum-PTGA gave a distinct precipitin test with AsBGG. When 4·5 ml of the serum were incubated with 0·5 mg AsBGG, a precipitate was obtained which was not noticeably soluble on addition of 0·5 ml of a 2% solution of AsBGG. The insoluble residue, after washing, weighed 2·2 mg; colorimetric comparison of its solution in 1% NaOH with a standard solution of AsBGG showed that it contained 0·2–0·3 mg AsBGG. When three 2 ml samples of the same immune serum were incubated with 0·2 mg PTA, PTGA or AsBGG, only the last substance gave a precipitate; it contained approximately 0·1 mg AsBGG.

We conclude from these results that poly-L-tyrosine-azophenylarsonate is not an antigen, but that poly-L-tyrosyl-gelatin-azophenylarsonate, if injected with alum as adjuvant, induces formation of precipitins which combine with the tyrosine-bound azophenylarsonate groups. Evidently, the nonantigenic poly-tyrosyl-azophenylarsonate acquires antigenic properties by its combination with gelatin.

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Department of Chemistry, Indiana University, Bloomington, Indiana, November 2, 1957.

Zusammenfassung

Polytyrosin und Polytyrosylgelatine wurden mit diazotierter Arsanilsäure gekoppelt und in Lösung oder nach Fällung mit Alaun und Alkali Kaninchen injiziert. Die injizierten Substanzen wurden von keinem der Sera präzipitiert. Hingegen präzipitierte das Serum der mit gefällter Arsanilazo-polytyrosyl-gelatine injizierten Tiere Arsanilazo-Rinderserumglobulin. Wir schliessen daraus, dass Arsanilazopolytyrosin nicht als Antigen wirkt, dass es aber durch Bindung an Gelatine antigene Eigenschaften gewinnt.

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Sucrose and Starch Synthesis in Sugar Cane Plant

During the investigations on the formation of sucrose in the sugar cane plant it was observed that all parts of the plant except the top node of the matured sugar cane contained only sucrose, glucose and fructose but not starch, at all stages of the development, whereas the top node of the matured sugar cane contained starch. This led us to think that a sucrose-synthesizing enzyme system may be predominant in all parts except the top node of the mature sugar-cane in which the presence of starch-synthesizing enzyme may be a special feature. Hence studies were undertaken to detect the sucrose- and the starch-synthesizing enzyme systems in various tissues of the plant at different stages of development and the results are recorded in this communication.

Young, middle-aged and matured sugar-cane plants were taken for the experiment. Cell-free extracts of leaves, roots, nodes and internodes were prepared as previously described[1] and tested for the presence of sucrose-synthesizing and starch-synthesizing enzyme systems.

For estimation of sucrose-synthesising enzyme system, 2 ml of the assay system contained citrate buffer (pH 6·5), 50 μM; fructose, 60 μM; glucose-1-phosphate,

[1] This work was supported by research grants of the National Science Foundation and the U.S. Public Health Service, and by contracts of Indiana University with the Office of Naval Research and the Atomic Energy Commission.

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[1] K. P. PANDYA and C. V. RAMAKRISHNAN, Naturwissenschaften *15*, 352 (1956).