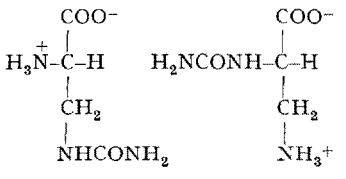
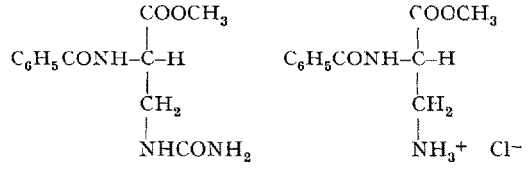


I II

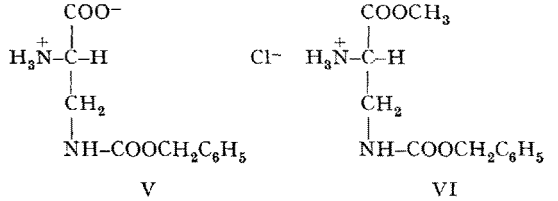
Upon benzoylation, albizziine[1,2] ($[\alpha]_D^{23}$: −67°C [c = 2·1 in H_2O]) afforded an oily N-benzoyl-derivative which was, in turn, esterified with diazomethane to yield the crystalline, *laevo*rotatory *N-benzoylalbizziine methyl ester* [m.p.[3] 171–174°C (from H_2O); Found: C 54·40; H 5·68; N 15·89. $C_{12}H_{15}N_3O_4$ requires C 54·33; H 5·70; N 15·84]. On critical comparison, this compound proved to be identical with a synthetic specimen of *methyl* L-*2-benzamido-3-ureidopropionate* (III) ($[\alpha]_D^{28}$: −32·5°C [c = 1·2 in CH_3OH]), prepared by reaction of methyl L-2-benzamido-3-aminopropionate hydrochloride[4] (IV) ($[\alpha]_D^{30}$: −48·3°C [c = 1·0 in CH_3OH][5]) with excess potassium cyanate in aqueous solution (pH 7) at 30°C.



III IV

The latter salt was synthesized according to SCHNEIDER[4], who further established its spatial relationship with the L-series by degradation to L-serine.

In course of the present studies, the albizziine-isomeride (II) was synthesized for comparison. L-2,3-Diaminopropionic acid hydrochloride ($[\alpha]_D^{24}$: +24·8°C [c = 4·7 in 1 N HCl]), isolated from seeds of *Mimosa Palmeri*[2], was treated with one equivalent of carbobenzoxy chloride in a phosphate buffer at pH 7 to give a 78% yield of a monosubstituted acid (V), separating from water in needles, m.p. 226–231°C (dec.) [Found: C 55·45; H 5·80; N 11·89. $C_{11}H_{14}N_2O_4$ requires C 55·45; H 5·92; N 11·76); ($[\alpha]_D^{21}$: −18·7°C (c = 1·0 in 1 N HCl), $[\alpha]_D^{18}$: −4·0°C (c = 1·1 in 0·1 N NaOH)]. The identity of the latter as L-*2-amino-3-carbobenzoxyamidopropionic acid* appeared from its transformation by means of diazomethane into a methyl ester hydrochloride, indistinguishable on basis of undepressed mixed melting point and coinciding infra-red spectra from an authentic sample of methyl L-2-amino-3-carbobenzoxyamidopropionate hydrochloride[4] ($[\alpha]_D^{21}$: −4·2°C [c = 2·1 in H_2O][5]).

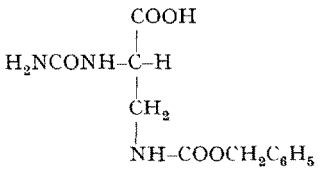


V VI

[3] Melting points are uncorrected and determined in an electrically heated block. Infra-red spectra (in KBr) have been determined of all the compounds discussed. Microanalyses were performed by Mr. P. HANSEN at the Chemical Laboratory of the University of Copenhagen.

[4] F. SCHNEIDER, Liebigs Ann. *529*, 1 (1937).

[5] No rotation data have formerly been published.



VII

Upon reaction with potassium cyanate, the acid (V) was transformed into the corresponding L-*2-ureido-3-carbobenzoxyamidopropionic acid* (VII), m.p 188–190°C (dec.) (from H_2O) [Found: C 51·10; H 5·58; N 15·01. $C_{12}H_{15}N_3O_5$ requires C 51·24; H 5·38; N 14·94]; ($[\alpha]_D^{17}$: −1·5°C [c = 1·0 in 0·1 N NaOH]), which on hydrogenolysis with Pd-black afforded L-*2-ureido-3-aminopropionic acid* (II), separating from aqueous ethanol in colourless needles, m.p. 204–210°C (dec.) [Found: C 32·75; H 6·24; N 28·70. $C_4H_9N_3O_3$ requires C 32·65; H 6·17; N 28·56]; ($[\alpha]_D^{25}$: +3·2°C [c = 1·0 in H_2O], $[\alpha]_D^{25}$: −43·0°C [c = 1·0 in 0·1 N HCl], $[\alpha]_D^{27}$: +24·6°C [c = 1·0 in 0·1 N NaOH]). Like albizziine, the isomeride (II) produces normal colour reactions with Ehrlich's reagent and ninhydrin, but differs from the former in other respects, notably in its infra-red spectrum and rotatory data.

Further results in connexion with studies of the degradation and synthesis of albizziine will form the subject of a forthcoming communication elsewhere.

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Organic Chemical Laboratory of the Royal Veterinary and Agricultural College, Copenhagen (Denmark), March 12, 1959.

Zusammenfassung

Eine neue Aminosäure, Albizziin, wurde durch chemische Verknüpfung zwischen N-Benzoylalbizziinmethylester und L-2-Benzamido-3-ureidopropionsäuremethylester (III) als L-2-Amino-3-ureidopropionsäure (I) identifiziert. Die Synthese von L-2-Ureido-3-aminopropionsäure (II) wird beschrieben.

The Metabolism of 3-Hydroxytyramine-1-C^{14} in Brain Tissue Homogenates[1]

Recently it was reported that the distribution of 3-hydroxytyramine in the brain is different from that of norepinephrine[2]. This finding suggests that 3-hydroxytyramine may have an independent role in brain function in addition to being a precursor of norepinephrine. While we have previously reported the metabolites of 3-hydroxytyramine in rats urine[3], to our knowledge no data on the metabolites of this compound recovered from brain tissue has been reported. We, therefore, investigated the metabolism of 3-hydroxytyramine in brain homogenates by the following procedure:

Fresh cow brain obtained from the slaughter house was homogenized in a Waring blender with ice cold phosphor

[1] This study was supported in part by United States Public Health Service Grant M 2717.

The authors are grateful to R. J. FLOODY, M. D. of Hoffmann-La Roche Inc., for providing us with iproniazid.

[2] A. BERTLER and E. ROSENGREN, Exper. *15*, 10 (1959).

[3] M. GOLDSTEIN, A. J. FRIEDHOFF, and C. SIMMONS, Biochem. Biophys. Acta (in press).

buffer of the composition: Krebs-Ringer-phosphate solution pH 7·4, 10 mg of glucose, adenosine triphosphate and diphosphopyridine nucleotide (all at a concentration of $5 \times 10^{-4} M$) – (incubate *A*). To a second preparation of identical composition 50 mg of iproniazid was added (incubate *B*). To each preparation 0·2 mg of 3-hydroxytyramine 1-C^{14} was added and the mixtures were incubated for 3 h at 37°, using air as a gas phase. At the end of the incubation the homogenates were deproteinized with 10% meta phosphoric acid and centrifuged. The residues were washed twice with 10% meta phosphoric acid and the combined aqueous supernatants were extracted three times with ethyl acetate. The combined ethyl acetate extracts were concentrated to a small volume *in vacuo* under nitrogen and an aliquot was chromatographed in descending chloroform-acetic acid-water (2:1:1) system overnight. The run off was chromatographed in isopropylalcohol-ammonia-water (8:1:1) over a period of 12 h[3]. The dry chromatograms were then scanned in a calibrated counting chamber, so that it was possible to locate all radioactive zones. The chloroform-acetic acid-water chromatograms from both incubates showed one radioactive peak which had the same mobility as authentic 3,4-dihydroxyphenylacetic acid. The isopropylalcohol-ammonia-water chromatograms from both incubates showed no radioactive zones, thus indicating the absence of 3-methoxy-4-hydroxyphenylacetic acid. The radioactive zone which had the same mobility as 3,4-dihydroxyphenylacetic acid was eluted with methylalcohol and rechromatographed in the following systems: (1) *n*-butanol-acetic acid-water (4:1:1); (2) benzene-propionic acid-water (100:7:5). In each of those chromatograms, the mobility of the radioactive zone was identical with authentic 3,4-dihydroxyphenylacetic acid. At this point, 30 mg carrier of 3,4-dihydroxyphenylacetic acid was added to the radioactive samples isolated from both incubates, and the samples were recrystallized from ethyl acetate-cyclohexane to constant specific radioactivity. The radioactivity in each stage of the purification of 3,4-dihydroxyphenylacetic acid is listed in Table I.

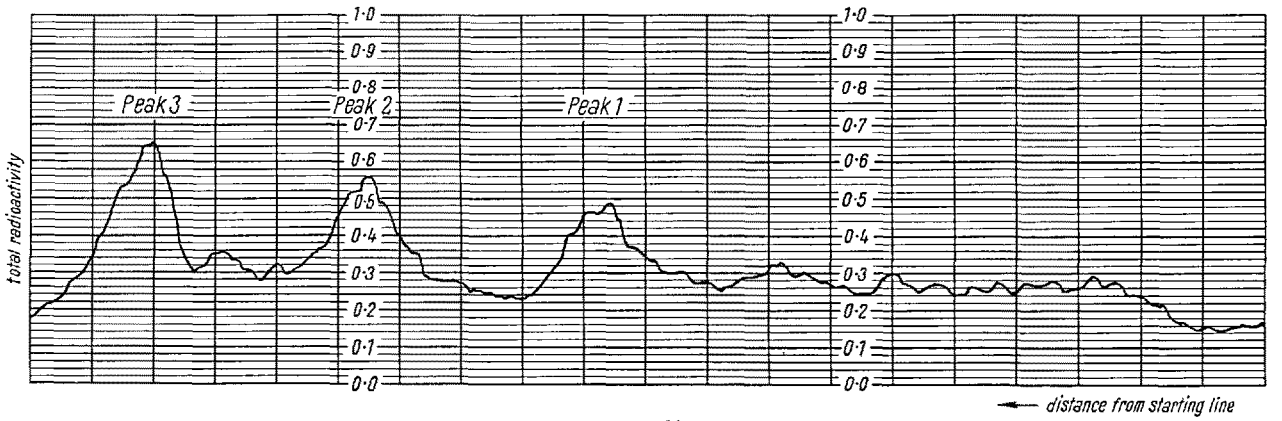
The aqueous layers from both incubates were adjusted to pH 4 and acetylated in the same manner as described in a previous publication[4]. After extracting the acetylated amines into methylene chloride, the radioactivity in each of those extracts was determined. The percentage of the total radioactivity recovered in the acetylated fraction was 14% for incubate *A* and 19% for incubate *B*. The methylene chloride extracts were concentrated *in vacuo*, and aliquots were applied on the paper and chromatographed in the Bush «C» system[5]. The descending chromatograms were developed after 4 h. This technique provided a satisfactory separation of acetylated catecholamines and methoxy-catechol amines and their mobility is listed in Table II. The dry chromatograms were scanned in a calibrated counting chamber and from each incubate three radioactive peaks were isolated (Fig.). In peak No. 1 was found 3% of the total radioactivity of the acetylated fraction from incubate *A* and 5·5% from incubate *B*. Peak No. 1 has the same mobility in the Bush «C» solvent system as epinephrine triacetate, but as yet has not been identified. In peak No. 2 was found 6% of the total radioactivity of the acetylated fraction from incubate *A*, and 7% from incubate *B*. Peak No. 2 has the same mobility as 3-hydroxytyramine triacetate. Upon acid hydrolysis it was deacetylated, and after an addition of 0·1 mg nonradioactive 3-hydroxytyramine as a carrier and chromatography in *n*-butanol/*n*-HCl, the radioactivity was found to be associated with 3-hydroxytyramine. In peak No. 3 was found 5% of the

Table I

The radioactivity of 3,4-dihydroxyphenylacetic acid, isolated from cow brain incubates in various stages of purification

State of purification	Incubate «A»		Incubate «B»*	
	Total radio activity C.p.m.	Specific activity C.p.m./mg	Total radio activity C.p.m.	Specific activity C.p.m./mg
Paper Chromatography:				
Chloroform-acetic acid-water	127,000		123,500	
n-butanol-acetic acid-water	130,000		126,000	
benzene-propionic acid-water	119,500		122,000	
Crystallization:				
First	93,000	3,100	87,000	2,900
Second	86,500	2,900	83,000	2,750
Third	84,000	2,800	81,500	2,700

* The concentration of iproniazid was 100 mg/kg of brain tissue. This dosage provided inhibition of monamine oxidase *in vivo*.



Paper chromatography separation of acetylated 3-hydroxytyramine-1-C^{14} and its amine metabolites in Bush chromatography 'C' solvent system.

[4] M. Goldstein, A. J. Friedhoff, and C. Simmons, Exper. *15*, 80 (1959).

[5] J. E. Bush, Biochem. J. *1951*, 370.

Table II

The mobilities of catecholamine triacetate derivatives and methoxycatecholamine diacetate derivatives in Bush «C» chromatography system*

Acetate derivate of:	Distance from starting line in cm	Mobility in cm/h
Norepinephrine	7–9	2
Methoxynorepinephrine .	11–13	3
Epinephrine	18–20	4·75
Methoxyepinephrine . . .	20–22	5·25
3-hydroxytyramine . . .	23–25	6
3-methoxytyramine . . .	27–29	7

* Descending technique with Whatman No. 1 filter paper prewashed with benzene: methylalcohol (1:1) was used. The catecholamines were developed by passing a narrow strip through a solution of 1% $FeCl_3$ and 2% potassium-ferricyanide, blotting and then passing it through a solution of 10% KOH in methylalcohol:water (1:1). The methoxy catecholamines were developed by passing a narrow strip through a solution of 0·1% dichloroquinone chlorimide in alcohol, blotting and then passing it through a solution of 0·1 *M* borate buffer pH 10, blotting again and exposing the strip to ammonia vapors.

total radioactivity of the acetylated fraction from incubate *A* and 6·5% from incubate *B*. Peak No. 3 has the same mobility as 3-methoxytyramine diacetate. After addition of 0·1 mg nonradioactive 3-methoxytyramine diacetate and rechromatography in the Bush «C» solvent system, the radioactive zone was eluted and treated with acylase from hog kidney at 37°C for 3 h. This removed the O-acetyl group from 3-methoxytyramine diacetate. The removal of the O-Acetyl group was demonstrated by the development of an olive color upon treatment with dichloroquinone chlorimide in alcohol and borate buffer, pH 10. This reaction is specific for the free phenolic group. The N-acetyl 3-methoxytyramine was then chromatographed in *n*-butanol acetic acid-water solvent system, and the radioactivity was found to be associated with this compound.

The present investigation demonstrates that 3·4 dihydroxyphenylacetic acid is the main metabolite of 3-hydroxytyramine in brain tissue. Although our results are based on the metabolism of 3-hydroxytyramine in cow brain homogenates, a preliminary study of brain homogenates obtained from rats shows the same pattern. The small differences in the radioactivity of the amine fraction of incubates *A* and *B* indicate that iproniazid has no significant effect as a monamine oxidase inhibitor in whole brain tissue homogenates. The isolation of 3-methoxytyramine indicates that some O-methylation of the intact amine occurs. It is of interest that in urine of rats the main metabolite of 3-hydroxytyramine was found to be 3-methoxy-4-hydroxyphenylacetic acid and not 3-4-dihydroxyphenylacetic acid[3]. These excretion studies demonstrate that 3-hydroxytyramine is metabolized by both O-methyl transferase and amine oxidase. However, from the present investigation it is evident that amine oxidase alone accounts for most of the metabolites of 3-hydroxytyramine in the brain.

Though the role of the corpus striatum in the etiology of Parkinson's disease is not completely understood, the presence of 3-hydroxytyramine in this area of the brain suggests that it may be related to this syndrome[2]. There are clinical reports[6], which indicate that reserpine can produce Parkinson like symptoms and reserpine is known to cause depletion of 3-hydroxytyramine from the brain[7]. This suggests that either the depletion of 3-hydroxytyramine or the activity of its metabolites may be related to Parkinson's syndrome. The possible relationship of the metabolites of 3-hydroxytyramine to Parkinson's disease is being investigated.

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Zusammenfassung

Homogenate aus Gehirn wurden mit 3-Hydroxytyramine-1-C^{14} inkubiert. Als Stoffwechselprodukt wurde aus der sauren Fraktion radioaktive 3,4-Dioxyphenylessigsäure isoliert. Aus der basischen Fraktion wurden drei radioaktive Zonen papierchromatographisch isoliert. Die erste Zone konnte noch nicht identifiziert werden. Die zweite wurde als 3-Hydroxytyramin und die dritte als 3-Methoxytyramin erkannt. Die mögliche Rolle der Stoffwechselprodukte von 3-Hydroxytyramin beim Parkinson-Syndrom wird diskutiert.

[6] S. KLINE, *Clinical Applications of Reserpine*, in *Psychopharmacology* (éd. N. S. KLINE, published by American Association for the Advance of Science, Washington, D. C. 1956), p. 81–108.

[7] A. CARLSON, E. ROSENGREN, A. BERTLER, and J. NILSSON, *Psychotropic Drugs* (Eds. S. GARATTINI and V. GHETTI, published by Elsevier Publishing Company, Amsterdam 1957), p. 363.

Note added in Proof: Peak No. 1 has also been shown to have similar mobility as acetylated 3,4,5-trihydroxyphenylethylamine.

The Authors wish to thank Dr. S. UDENFRIEND, National Institute of Health, Bethesda, Md., for providing this compound.

Messung der Serum-Insulin-Aktivität mit epididymalem Ratten-Fettgewebe *in vitro*

MARTIN[1] beschrieb eine Bestimmungsmethode für Serum-Insulin-Aktivität mit epididymalem Fettgewebe *in vitro*, wobei die $C^{14}O_2$-Produktion aus 1-C^{14}-Glukose als Index für das vorhandene Insulin benützt wird. Aus unserem Labor berichtete STAUB[2] über eine Nachweismethode für Insulin mit Hilfe der Glukoseaufnahme von epididymalem Rattenfettgewebe *in vitro*. Modifikationen seiner Technik erlauben es uns jetzt, die Serum-Insulin-Aktivität zuverlässig, konstant und relativ spezifisch zu messen.

Methodik. Als Versuchstiere dienten männliche Albinoratten (Spitalstamm), ernährt mit NAFAG-Rattenfutter und Wasser *ad libitum*, von einem Gewicht von 120–160 g.

Als Inkubationsmedium wurde Krebs-Ringer-Bicarbonat-Puffer[3] verwendet, pH 7,4, mit Zusatz von 200 mg% Gelatine und 200 mg% Glukose. Die Pufferlösung wurde für jeden Versuch aus Stammlösungen frisch zubereitet.

[1] D. B. MARTIN, A. E. RENOLD und Y. M. DAGENAIS, Lancet *II/1958*, 76.

[2] M. STAUB, Schweiz. med. Wschr. *88*, 831 (1958).

[3] W. W. UMBREIT, R. H. BURRIS und J. F. STAUFFER, *Manometric Techniques and Related Methods for the Study of Tissue Metabolism* (Minneapolis 1945), p. 194.