Brit. J. Pharmacol. (1948), **3**, 315.

THE DECARBOXYLATION OF β-3 : 4-DIHYDROXYPHENYLSERINE (NORADRENALINE CARBOXYLIC ACID)

BY

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(Received June 17, 1948)

β-3 : 4-Dihydroxyphenylserine is an amino-acid structurally closely related to noradrenaline. In this paper it will therefore be called "noradrenaline carboxylic acid."

The amino-acid was first prepared by Rosenmund and Dornsaft in 1919, and again by Guggenheim (1940), who found that in rabbits it was without action on the arterial blood pressure after subcutaneous injection of 0.1 g./kg. The acid has recently been prepared by Dalgleish and Mann (1947), together with the corresponding N-methyl-amino-acid, "adrenaline carboxylic acid."

Rosenmund and Dornsaft (1919) suggested that the amino-acid might be a precursor of adrenaline in mammals, but they did not support this suggestion by experiments.

A scheme of adrenaline synthesis has been discussed which assumes the decarboxylation of *L*-3 : 4-dihydroxyphenylalanine (Blaschko, 1939, 1942, 1948), a reaction catalysed by the enzyme dopa decarboxylase (Holtz, Heise, and Lüdtke, 1938). This enzyme does not act on N-methyl-3 : 4-dihydroxyphenylalanine (Blaschko, 1939), but it is not known how the introduction of a hydroxyl group in the β-position affects substrate specificity. We have therefore examined noradrenaline carboxylic acid as a possible substrate of mammalian decarboxylases.

The mammalian dopa decarboxylase is related to the bacterial *L*-tyrosine decarboxylase, and we have therefore included experiments on the decarboxylation of the amino-acid by the bacterial enzyme; these experiments have enabled us to establish the stereochemical configuration of 3 : 4-dihydroxyphenylserine.

MATERIALS AND METHODS

For the noradrenaline carboxylic acid we are grateful to Dr. F. G. Mann, F.R.S. Two samples of noradrenaline hydrochloride were used for the biological assay; the first was a racemic preparation, known as Arterenol, which was obtained from the I.G. Farben-Industrie more than ten years ago; for the second sample, a specimen of the laevorotatory stereoisomer, we are grateful to Dr. M. L. Tainter.

The tissue extracts—from guinea-pig's kidney and suprarenal gland—were prepared as previously described (Blaschko, 1942). All our manometric experiments were conducted in an atmosphere of nitrogen.

An acetone-dried preparation was used for the study of the bacterial *L*-tyrosine decarboxylase. We are grateful to Prof. I. C. Gunsalus for a strain of *Streptococcus faecalis* R (ATCC 4083); the bacteria were grown on a medium similar to that described by Bellamy and Gunsalus (1945), but with an addition of 0.5 mg./litre of pyridoxal (Merck).

The pharmacological assay was carried out on the arterial blood pressure of the spinal cat; the rat's uterus preparation used in one experiment has been described by Garcia de Jalon, Bayo Bayo and Garcia de Jalon (1945).

EXPERIMENTS

(1) *Experiments with guinea-pig's kidney extract*

The guinea-pig's kidney gives extracts of high dopa decarboxylase activity; we compared the rate of decarboxylation of the racemic 3 : 4-dihydroxyphenylserine with that of *DL*-3 : 4-dihydroxyphenylalanine. The manometric set-up was as follows:

	Flask 1	*Flask* 2	*Flask* 3
Main Flask	1.6 ml. guinea-pig kidney extract (in all flasks)		
Side Bulb	0.4 ml. water	0.4 ml. M/100 *DL*-3 : 4-dihydroxyphenylalanine	0.4 ml. M/100 noradrenaline carboxylic acid

Incubation temperature 37.5° C.

From one molecule of *DL*-dihydroxyphenylalanine half a molecule of carbon dioxide should be formed, as it is known that the decarboxylase is stereospecific and will form carbon dioxide only

from the *L*-isomer. The amount of carbon dioxide expected to be formed from 0.4 ml. of M/100 *DL*-amino-acid is 44.8 μl.

From 3:4-dihydroxyphenylalanine the theoretical amount of carbon dioxide was formed within the first nine minutes, and the reaction then came to a standstill. There was no formation of carbon dioxide from noradrenaline carboxylic acid.

(2) *Experiments with extracts from the guinea-pig's suprarenal gland*

There was no formation of carbon dioxide when extracts from the suprarenal gland were incubated with either 3:4-dihydroxyphenylalanine or noradrenaline carboxylic acid.

(3) *Experiments with* Streptococcus faecalis

It is known that the *L*-tyrosine decarboxylase of *Strep. faecalis* also acts on 3:4-dihydroxyphenylalanine (Epps, 1944). It seemed therefore of interest to find out how the introduction of a hydroxyl group in the β-position of the side chain would affect substrate specificity.

Two experiments were carried out in which the amino-acid was incubated with the bacterial preparation. Carbon dioxide was formed in both experiments. This suggested that noradrenaline had been formed by the enzyme. The pressor activity of the solutions was therefore estimated on the blood pressure of the spinal cat. In the first experiment the activity was assayed against (±)-noradrenaline and in the second against the laevorotatory isomer. Since the results obtained in the two experiments showed satisfactory agreement, only the second experiment will be described in detail.

(*a*) *Incubation.*—A suspension was prepared containing 10 mg. of the acetone-dried bacteria in 1 ml. of distilled water.

Two manometer flasks were set up as follows:

	Blank	*Test*
Main Flask	1.0 ml. bacterial suspension + 0.1 ml. M/1 acetate buffer, *p*H 5.5 + 0.2 ml. water in both flasks	
Side Bulb	0.2 ml. water	0.2 ml. M/12.5 noradrenaline carboxylic acid

A third flask was set up in which the noradrenaline carboxylic acid in the side bulb was replaced by 0.2 ml. of a suspension of *L*-tyrosine. This flask was used in order to enable us to relate the rate of decarboxylation of noradrenaline carboxylic acid to that of tyrosine.

The incubation was carried out at a temperature of 28.5° C.

Separately, a smaller amount of *L*-tyrosine was incubated with the bacterial preparation under the same conditions; this was done in order to determine how much of the carbon dioxide formed had been retained as bicarbonate. The retention was found to amount to about 6 per cent; this figure was therefore used as a correction in the main experiment.

Formation of carbon dioxide occurred when noradrenaline carboxylic acid was added to the enzyme. The rate of decarboxylation was steady for the first hour, but then slowed down; the reaction had almost come to a standstill when the incubation was stopped after 315 min. By that time 168 μl. of carbon dioxide had been formed. On the assumption that half a molecule of carbon dioxide is formed from one molecule of noradrenaline carboxylic acid 179 μl. should be formed. Thus the observed formation of carbon dioxide represents 94 per cent of theory. The time course of the reaction is shown in Fig. 1.

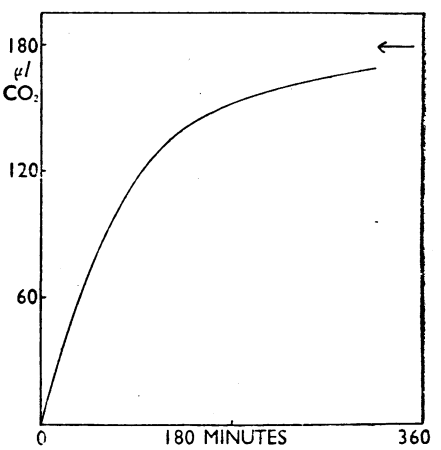


FIG. 1.—Decarboxylation of noradrenaline carboxylic acid by *Streptococcus faecalis* R. Abscissa: time in minutes; Ordinate: μl. CO_2 formed. The arrow denotes half a molecule of CO_2 per molecule of amino-acid added.

After the last reading the contents of the blank and test flasks were each pipetted into a centrifuge tube. Each flask was washed out with two successive portions of 0.25 ml. of water. For the pharmacological assay it was assumed that any active material formed was now contained in a total volume of 2.0 ml.

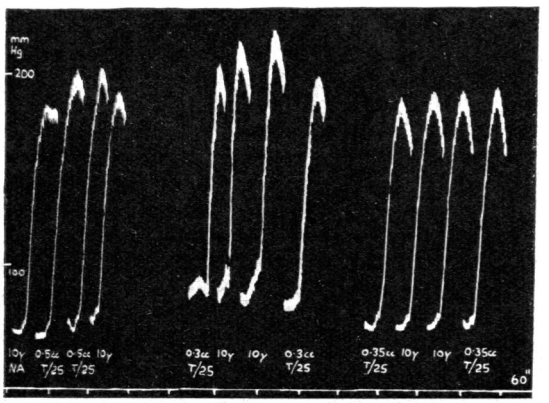
The suspensions were centrifuged for 15 min., in order to remove the bacterial debris, and the supernatant fluids were used for the assay.

(*b*) *Assay.*—The manometric experiment suggested that amine formation from noradrenaline

carboxylic acid had occurred. It was desirable to establish the identity of the amine formed with noradrenaline and to determine its pharmacological activity. The assay was carried out on the spinal cat and the identity of the pressor principle was confirmed on the rat's uterus.

Spinal cat; 3.2 kg.; ♂. The test was used in a dilution 1 in 25. The standard of laevorotatory noradrenaline was made up in a solution of the blank so that the final concentration of the blank was also 1 in 25.

The pressor activity of the test sample is shown in Fig. 2; the tracing shows that 0.5 ml. of the test,



(*a*) (*b*) (*c*)

FIG. 2.—Assay of pressor activity produced from noradrenaline carboxylic acid by the bacterial enzyme. Arterial blood pressure of the spinal cat. 10 μg. of synthetic (−)-noradrenaline were: (*a*) less active than 0.5 ml., (*b*) more active than 0.3 ml., (*c*) about as active as 0.35 ml. of the test solution, diluted 1 in 25.

diluted 1 in 25, had a stronger, and 0.3 ml. of the same solution a weaker, pressor action than 10 μg. of (−)-noradrenaline. The pressor action of 10 μg. of (−)-noradrenaline was found to be approximately equal to that of 0.35 ml. of the test, diluted 1 in 25. The blank was without action on the blood pressure.

(*c*) *Calculation of result.*—The total amount of pressor activity formed in the experiment can be calculated from these data. The pressor action contained in 2 ml. of the undiluted test was equivalent to more than 1.00 mg. and less than 1.62 mg. of (−)-noradrenaline, and about equal to 1.40 mg. of (−)-noradrenaline.

The total amount of amine expected to be present can be calculated from the amount of carbon dioxide formed; one molecule of amine is formed per molecule of carbon dioxide:

$$R\text{-}CH(NH_2)COOH \longrightarrow R\text{-}CH_2NH_2 + CO_2$$

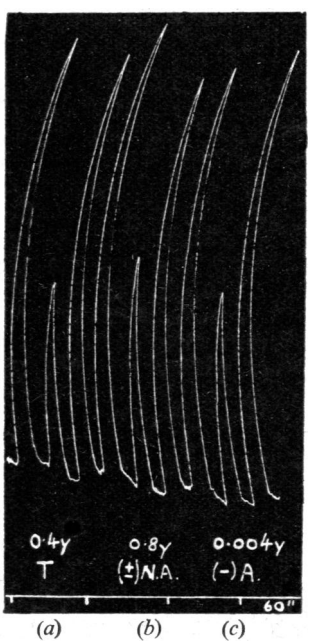
The molecular weight of noradrenaline is 169. Therefore, 22,400 μl. of CO_2 correspond to 169 mg. of noradrenaline, and 168 μl. of CO_2, the amount formed in the experiment, correspond to

$$\frac{169 \times 168}{22{,}400} = 1.27 \text{ mg. of noradrenaline.}$$

The agreement between the amounts found and expected is satisfactory and shows that the amine formed was in fact (−)-noradrenaline. The amine formed cannot have been (±)-noradrenaline.

Similarly, it could be calculated that in the first experiment, in which the activity was assayed against (±)-noradrenaline, the amount of amine formed was about twice as active as (±)-noradrenaline. In this experiment the assay on the cat's blood pressure was followed by an assay on the rat's uterus.

(*d*) *Rat's uterus assay.*—One horn of the uterus of a non-pregnant rat was suspended in a bath of capacity 5 ml. at a temperature of 31° C. The composition of the Ringer's fluid was similar to that recommended by Garcia de Jalon *et al.* (1945). Submaximal contractions of the uterus were recorded; a dose of 5 μg. of acetylcholine was added to the bath every 90 secs. The acetylcholine was present for 30 secs., and 60 secs. were allowed for recovery. Adrenaline, noradrenaline, or the test solution was added to the bath 30 secs. before the acetylcholine.



(*a*) (*b*) (*c*)

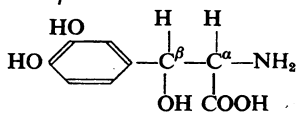
FIG. 3.—Isolated uterus of the rat. Each contraction was caused by 5 μg. of acetylcholine. The inhibitions were due to: (*a*) the test solution in an amount calculated to contain 0.4 μg. of amine, (*b*) 0.8 μg. of (±)-noradrenaline, (*c*) 0.004 μg. of (−)-adrenaline.

A dilution of the test was given calculated to contain 0.4 μg. of (−)-noradrenaline. Fig. 3 shows that the inhibition observed was about equal to that after 0.8 μg. of (±)-noradrenaline. The same result was obtained at three different dose levels. The Figure confirms West's (1947) finding that the rat's uterus preparation is many times more sensitive to adrenaline than to noradrenaline.

The result obtained on the rat's uterus preparation therefore confirms that of the blood pressure assay: the amine formed is (−)-noradrenaline.

Discussion

In order to assess whether or not noradrenaline carboxylic acid can be considered as a likely precursor of adrenaline or sympathin, it is necessary to consider its structure. The acid differs from 3:4-dihydroxyphenylalanine in that it contains 2 asymmetric carbon atoms, one in position α and one in position β.



Noradrenaline carboxylic acid

Compounds of this kind have four different configurations and exist in pairs related as object and mirror image. The four possible arrangements in space are shown in Fig. 4, in which the α and β carbon atoms are replaced by regular tetrahedra. The designations *L* and *D* are used for the α carbon atom: the configuration *L* is that common to most naturally occurring amino-acids. The two possible configurations of the β carbon atom are distinguished as *l* and *d*; this is the asymmetric carbon atom of adrenaline; the naturally occurring laevorotatory configuration is usually referred to as *l*-adrenaline.

Dr. Mann told us that the sample of noradrenaline carboxylic acid which we used in our experiments behaved like a simple racemic mixture and that it therefore contained only one pair of stereoisomers. The two pairs of stereoisomers are *Dd–Ll* and *Dl–Ld*.

The experiments reported above enable us to determine the configuration of noradrenaline carboxylic acid. This is possible, because two stereospecific affinities are involved:

(*a*) that of the bacterial amino-acid decarboxylases for the *L*-configuration of the α carbon atom, and

(*b*) that of the plain muscle cell for the *l*-configuration of the β carbon atom.

All known amino-acid decarboxylases, bacterial and mammalian, are stereospecific, and it can therefore be safely assumed that only the *L*-stereoisomer was decarboxylated in our experiments. This is borne out by the observation that approximately half a molecule of carbon dioxide was formed from one molecule of noradrenaline carboxylic acid.

The stereospecificity of the mammalian receptors has recently been studied by Tainter, Tullar, and Luduena (1948), who have shown that on the spinal cat's blood pressure the ratio of equiactive doses of laevorotatory to dextrorotatory noradrenaline is 1:25 to 1:33.

Theoretically three structures are possible:

(1) a racemic mixture of the configurations *Dd* and *Ll*; in this case one would expect (−)-noradrenaline to be formed.

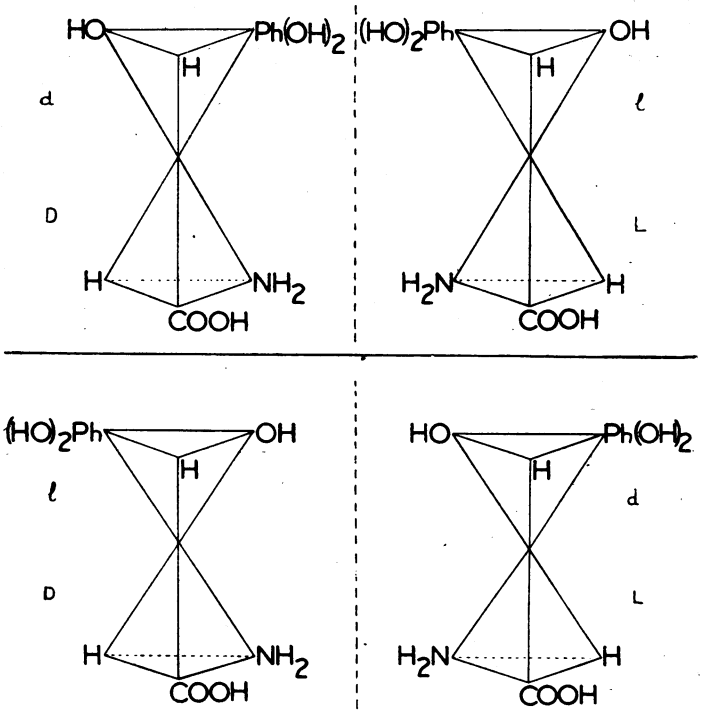


FIG. 4.—The four configurations of "noradrenaline carboxylic acid." The alpha carbon atoms are represented by the lower, and the beta carbon atoms by the upper, tetrahedra.

(2) a racemic mixture of the configurations *Dl* and *Ld*; in this case one would expect (±)-noradrenaline to be formed.

(3) a mixture of all four configurations; in this case one would expect (±)-noradrenaline to be formed. (This possibility was already ruled out by Dr. Mann's findings.)

That the first of these is in fact the structure of the acid is proved by the pharmacological assay on the spinal cat. The *L*-amino acid was found to be almost quantitatively decarboxylated and to have given rise to (−)-noradrenaline.

Rules for the nomenclature of amino-acids have recently been agreed upon by the editors of the *Biochemical Journal*, the *Journal of the Chemical Society*, and the corresponding American publications (1948). Rule 6 deals with amino-acids "with two asymmetric centres, but where internal compensation is impossible." Noradrenaline carboxylic acid is unlikely to be present in proteins, but it is so closely related to amino-acids found in proteins that it seems desirable to follow the rules laid down for these compounds. The substance studied in this paper contains the configuration in which the *L*-amino-acid has the configuration related to naturally occurring laevorotatory adrenaline, and it is therefore proposed to use the designation 3:4-dihydroxyphenylserine for this specimen and to reserve the prefix allo- for the pair which still awaits synthesis.

3:4-Dihydroxyphenylserine has the configuration to be expected in a possible precursor of adrenaline, so that the negative results obtained with the mammalian decarboxylases cannot be ascribed to the use of the wrong stereoisomer but must be interpreted as meaning that this substance is unlikely to be a precursor of adrenaline or sympathin.

Summary

(1) Noradrenaline carboxylic acid is not a substrate of dopa decarboxylase.

(2) Extracts of guinea-pig's suprarenal glands do not contain dopa decarboxylase, and they do not decarboxylate noradrenaline carboxylic acid.

(3) Noradrenaline carboxylic acid was decarboxylated by an acetone-dried preparation of *Streptococcus faecalis* R; the amine formed was characterized as (−)-noradrenaline by pharmacological assay on the blood pressure of the spinal cat and on the rat's uterus.

The authors are grateful to the Medical Research Council for a personal grant to one of us (G. H. S. S.).

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