Chapter 38

The Basic Constituents of Toad Venoms

VENANCIO DEULOFEU AND EDMUNDO A. RÚVEDA

DEPARTAMENTO DE QUÍMICA ORGÁNICA, FACULTAD DE CIENCIAS EXACTAS Y NATURALES, AND CÁTEDRA DE FITOQUÍMICA, FACULTAD DE FARMACIA Y BIOQUÍMICA, UNIVERSIDAD DE BUENOS AIRES, ARGENTINA

I. INTRODUCTION

The secretion of the parotoid glands of the toads, usually known as toad venom, contains two principal classes of pharmacologically active constituents. One class is formed by substances belonging to the steroids, the bufadienolides (bufogenins) and their derivatives, the bufotoxins (Chapter 40). To the other class belong different basic compounds. In some cases these compounds have been isolated from excised parotoid glands, in other cases the whole skin, including adhering parotoid and other small glands, has been extracted. Finally, the secretion of the glands has been collected and used as prime material for the extraction.

Although slight differences have been described, for some species, in the bases present in the glands, the skin, or the secretion, we will consider in this chapter the basic substances derived from all three sources and use for them the general denomination of toad venoms.

We also include information on the secretion of the glands used in China, in popular medicine, with the name of Ch'an Su, known in Japan as Senso, and which seems to be prepared from *B. gargarizans*.

II. HISTORY

The presence in the secretion of the parotoid glands of basic substances giving a positive alkaloid reaction was detected by Phisalix and Bertrand in 1893, working with the European toad *Bufo bufo bufo* (*B. vulgaris*). They obtained an amorphous base which was named bufotenine (Phisalix and Bertrand, 1902).

In 1912, Abel and Macht isolated crystalline adrenaline from the secretion of *B. marinus*, a widely dispersed American tropical toad. In 1920, Handovsky crystallized the oxalate and the picrate of a base present in the secretion of *Bufo bufo bufo* and kept for it the name of bufotenine, given earlier by Phisalix and Bertrand to the amorphous preparation.

From that time studies on the distribution, characterization, isolation, and determination of the chemical structure of the basic constituents present in the toad venoms were carried out in several laboratories.

The venoms of the genus *Bufo* contain bases of two different chemical types: (a) derived from phenylethylamine, (b) derived from tryptamine.

It was made clear after several species were studied that although there were differences in the bases that could be isolated from each, one could hardly speak of species specificity.

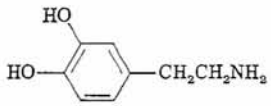
The systematic application of different methods of chromatography has widened our knowledge of the distribution of the bases in many species of toads but has not clarified our understanding of the biological significance of them, and the role they play in the animals.

Adrenaline was the first crystalline base isolated and for many years the only representative of the bases of phenylethylamine type. That other bases were indolic was suspected in their early chemical studies by H. Wieland *et al.* (1932) and by Jensen and Chen (1932). For the older literature see Tschesche (1945), Deulofeu (1948), and Kaiser and Michl (1958).

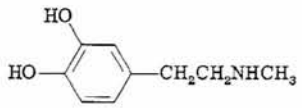
III. THE PHENYLETHYLAMINE BASES

A. Introduction

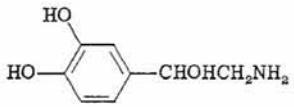
The following bases derived from phenylethylamine have been identified in toad venoms: dopamine (I), *N*-methyldopamine (epinine) (II), noradrenaline (norepinephrine) (III), adrenaline (epinephrine) (IV). All belong to the class of the catecholamines.



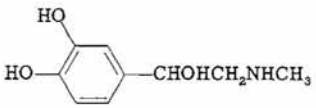
(I)



(II)



(III)



(IV)

B. Distribution

The distribution of the catecholamines in venoms from different species of toads is given in Table I.

Only adrenaline (IV) has actually been isolated. It was found to be the

TABLE I

CATECHOLAMINE BASES FOUND IN TOAD VENOMS[a]

Dopamine (I) HCl, mp, 240°–241°C; picrate, mp 189°C (Barger and Ewins, 1910; Wasser and Sommer, 1928).
Identification: *B. marinus* (Märki *et al.*, 1962).

N-Methyldopamine; Epinine (II) mp 186°–187°C, HCl, mp 177°–178°C (Bretschneider, 1947).
Identification: *B. marinus* (Märki *et al.*, 1962).

(—)-Noradrenaline; (—)-Norepinephrine (III) mp 216.5°–218°C $[\alpha]_D^{25°}$–37.3°C (H_2O,1 equiv. HCl); HCl, mp 145.2°–146.4°, $[\alpha]_D^{25°}$–40.0°C (H_2O) (Tullar, 1948).
Identification: Ch'an Su, (Lee and Chen, 1951), *B. marinus*. (Lasagna, 1951; Märki *et al.*, 1962). 0.20 μg per gram of glands (Östlund, 1954).

(—)-Adrenaline; (—)-epinephrine (IV) mp 216°–218°C; $[\alpha]_D^{20°}$–50.7°C (HCl,H_2O).
Identification: *B. marinus*, 5–7% in secretion (Abel and Macht, 1911–1912), 6–11.6% (Fischer and Lecomte, 1950) (Märki *et al.*, 1962). *B. regularis*, 4.6% in secretion (Chen and Chen, 1933). *B. arenarum*, 5.1% in glands, *B. mauretanicus*, 1% (Fischer and Lecomte, 1950). *B. vulgaris*, 3.7 μg per gram of glands (Östlund, 1954). *B. formosus* (S. Ohno and Komatsu, 1957). *B. crucifer* (Pereira and de Oliveira, 1961).
Isolation: B. marinus, 4.5% dried secretion (Abel and Macht, 1911–1912); 1.35% *idem* (Slotta *et al.*, 1937), Ch'an Su (Jensen and Chen, 1929; Chen *et al.*, 1931); *B. arenarum* (Deulofeu, 1935), 4 mg/gm dried secretion (Jensen, 1935); *B. regularis*, 3 mg/gm (Jensen, 1935); *B. paracnemis* (Deulofeu and Mendive, 1938).

[a]Tables I and II give information on the distribution of catecholamines (Table I) and indolethylamines (Table II) in the genus *Bufo*. Information on species belonging to other genera is sometimes added for comparison. The data include results from skins, excised parotoid glands, and secretions.

Isolation means that the base or at least one derivative, has been isolated in crystalline condition. Under identification, the data quoted have been obtained either by pharmacological methods or, mainly in recent years, by paper chromatography, and in those papers, information of interest, especially about systems of solvents and developing reagents, is found.

same stereoisomer obtained from the adrenal of higher animals: R-(—)-adrenaline (Pratesi *et al.*, 1958).

Noradrenaline (III) has been detected pharmacologically, chemically, and by paper chromatography. Lasagna (1951) determined by chemical reactivity and by paper chromatography that it was present in the old preparation of adrenaline from Abel and Macht (1911-1912) which derived from *B. marinus*. It has been detected in the extract from excised parotoid glands and from the secretion of the same species by Märki *et al.* (1962). Also, in an old preparation of adrenaline from Ch'an Su, Lee and Chen (1951) detected noradrenaline pharmacologically. The method employed verifies also that it is the same stereoisomer found in higher animals: R-(—)-noradrenaline (Pratesi *et al.*, 1959).

N-Methyldopamine (II) and dopamine (I) have been detected by paper chromatography in *B. marinus* (Märki *et al.*, 1962), the former for the first time in animals. It is evident that we can expect the finding of these catecholamines in other species of toads by the use of methods of greater sensibility, like those employed in this particular case.

Adrenaline (IV) and noradrenaline (III) (von Euler, 1956) are widely distributed in animals where they play the role of hormones. Dopamine (I), which is a biochemical precursor of noradrenaline, has also a large distribution.

On the other hand *N*-methyldopamine (II) has only been found in the venom of *B. marinus* and not in higher animals, although in them it can be enzymically transformed into adrenaline (Bridgers and Kaufman, 1962).

With the exception of adrenaline, the remaining catecholamines have also been found in products of vegetable origin. Noradrenaline and dopamine are the most common (Udenfriend *et al.*, 1959). The banana is a fruit containing usually rather large amounts of them (Waalkes *et al.*, 1958). *N*-Methyldopamine has been found in extracts of *Spartium scoparium* (Jaminet, 1959) and seeds of *Vicia faba* (Piccinelli, 1955).

C. Biosynthesis

A considerable amount of work has been done on the biosynthesis and metabolism of the catecholamines in higher animals (for reviews see Axelrod, 1959; Daly and Witkop, 1963; Weiner, 1964).

On the other hand, no data is available on their formation in toads. Pending further experimental work it can be assumed that in toads, the

production of catecholamines follows, if not exactly the same, similar paths as those found in other animals, although species differences could be expected.

In higher animals the catecholamines are produced following the paths indicated in Fig. 1.

Phenylalanine (V) and tyrosine (VI) are the starting points of their biosynthesis, an intermediate being 3,4-dihydroxyphenylalanine (dopa, VIII) which on enzymic decarboxylation produces dopamine (I). This in turn can

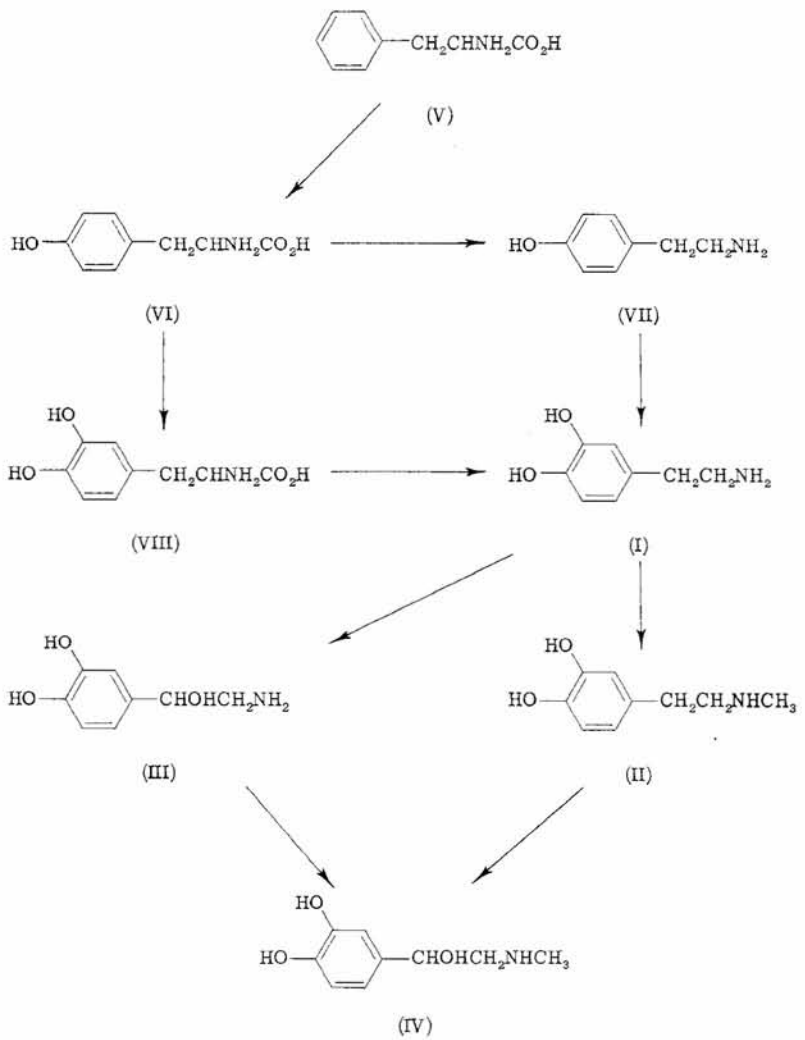


FIG. 1. Biosynthesis of the catecholamines.

be *N*-methylated to *N*-methyldopamine (II) or hydroxylated to noradrenaline (III). Oxidation of *N*-methyldopamine (II) or *N*-methylation of noradrenaline, gives in both cases adrenaline (IV). A minor pathway in the formation of dopamine (I) is tyramine (VII), formed by decarboxylation of tyrosine (VI).

It is interesting that all the four catecholamines (I–IV) involved in the metabolic pathways indicated in Figure I have been found in toad venoms. Besides, the work of Märki *et al.*, (1962) on the venom of *B. marinus*, has shown that it contains two enzymes which play a role in the *N*-methylation of catecholamines. One is a phenylethanolamine *N*-methyl transferase, which catalyses the *N*-methylation of phenylethanolamines by employing *S*-adenosylmethionine as methyl donor. It is similar, although not identical, to an enzyme found in adrenal glands (Axelrod, 1962). The other is an unspecific *N*-methyl transferase, which uses the same methyl donor and methylates a series of phenylethylamines and tryptamine derivatives. It is similar in action to an enzyme described by Axelrod (1961, 1963) present in rabbit lungs.

D. Metabolism

In man and higher animals the metabolism of noradrenaline (III) and adrenaline (IV) follows very similar pathways, the main steps being indicated in Fig. 2. The importance of some of the metabolic steps varies with the species and leads to differences in the main products excreted.

There are two interesting aspects to this metabolism. One is the oxidation of the side chain, initiated by a monoamine oxidase, which in the case of noradrenaline (III) produces an aldehyde; the latter in man, by further oxidation forms mandelic acid (XII) which with its 3-*O*-methyl ether (X) are the main products excreted. In rats, the aldehyde is reduced to the glycol (XIII), which on *O*-methylation produces the ether (XIV), an important metabolite in that species. Vanillic acid (XI), a further product of oxidation, has been found to be also a metabolic product of noradrenaline in man (Rosen and Goodall, 1962).

The other aspect is the enzymic *O*-methylation of the phenolic group at carbon 3. This methylation is catalyzed by a catechol *O*-methyl transferase which is widely distributed in a variety of species and tissues. It can 3-*O*-methylate the catecholamines and many of their metabolites. The methyl group is transferred from *S*-adenosylmethionine and Mg^{++} is needed for its activity (Axelrod and Tomchick, 1958).

It is responsible for the direct or indirect production of several of the metabolites indicated in Fig. 2: 3-*O*-methylnoradrenaline (IX); 4-hydroxy-3-methoxymandelic acid (X); 4-hydroxy-3-methoxyphenylethyleneglycol (XIV) and vanillic acid (XI). (Axelrod *et al.*, 1958a,b). It is also responsible for the *O*-methylation of adrenaline (IV) to 3-*O*-methyladrenaline (XV), the metabolism of both bases being parallel to that of noradrenaline and 3-*O*-methylnoradrenaline, as can be seen in Fig. 2.

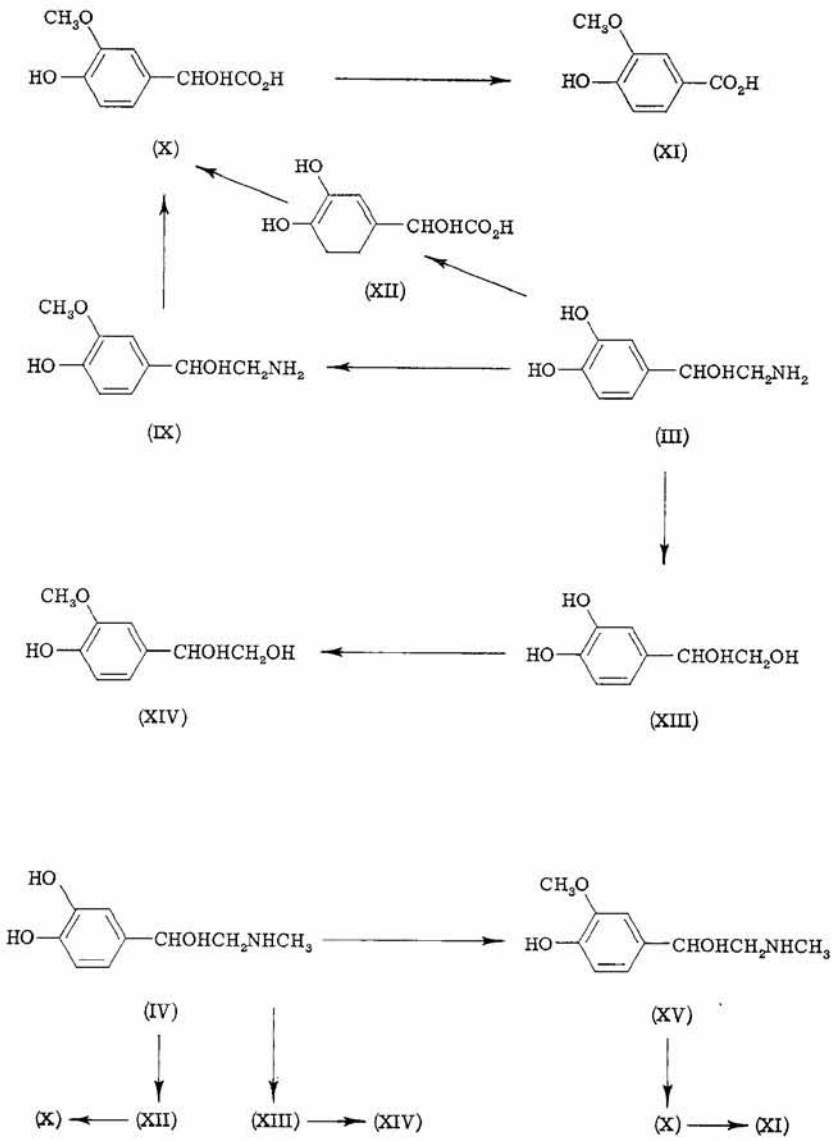


FIG. 2. Metabolic pathways of noradrenaline (III) and adrenaline (IV).

The metabolites of the catecholamines are usually excreted by the urine, although a minor excretion can also take place through the bile (rats). Several of the metabolites excreted are conjugated with glucuronic or with sulfuric acid (Hertting and La Brosse, 1962).

IV. THE TRYPTAMINE BASES AND DERIVATIVES

Introduction

The following bases derived from tryptamine have been isolated or identified with certainty from toad venoms, skins, or excised parotoid glands (Table II): (a) 5-hydroxytryptamine (serotonin) (XVI); (b) *N*-methyl-5-hydroxytryptamine (XVII); (c) *N*-methyl-5-methoxytryptamine (XVIII); (d) bufotenine (*N,N*-dimethylserotonine) (XIX) and its sulfuric acid ester, known as bufoviridine (XX); (e) *O*-methylbufotenine (XXI); (f) bufotenidine (*N,N,N*-trimethylserotonin) (XXII); (g) dehydrobufotenine (XXIII) and its sulfuric acid ester bufothionine (XXIV). The two last compounds are not indolethylamine bases but because of their chemical and biological relation to the former it is convenient to consider them together.

TABLE II

TRYPTAMINE BASES FOUND IN TOAD VENOMS

5-Hydroxytryptamine, serotonin, enteramine, thrombocytin, thrombotonin (XVI).
mp 212°–214°C (Rapport *et al.*, 1948); HCl, mp 167°–168°C (Hamlin and Fischer, 1951). Oxalate, mp 194°–196°C. Picrate, mp 196°–197°C (Harley-Mason and Jackson, 1954). Creatinine sulfate complex, mp 214°–216°C (Speeter *et al.*, 1951). UV spectrum: λ 274 mμ (logϵ 3.77), shoulder 293 (3.63) (H_2O, pH 5.3). At pH 11.9 a small shift is observed in the 274 maximun, the shoulder disappears and a new maximum is observed at 322 mμ (Rapport, 1949; Asero *et al.*, 1952).
Identification: B. marinus (Udenfriend *et al.*, 1952). *B. bufo bufo* (Spandrio, 1961), *B. americanus, B. arenarum, B. bergei, B. calamita, B. fowleri, B. gargarizans, B. kisoloensis, B. mauretanicus, B. regularis, B. viridis* (Erspamer, 1954, 1961). *B. alvarius*, 4–6 μg per gram of dried skin (Erspamer *et al.*, 1965).
Isolation: B. arenarum (Frydman and Deulofeu, 1961). In other genera: *Xenopus laevis* (van de Veerdonk *et al.*, 1961). *Bombinator pachypus, B. igneus, Discoglosus pictus, Hyla arborea, H. aurea, Rana esculenta, R. pipiens, R. palustris, R. madagascariensis, R. labrosa, Salamandra maculosa* (Erspamer, 1954, 1961).

N-Methyl-5-hydroxytryptamine (XVII). Oxalate, mp 153°–159°C (Stoll *et al.*, 1955).
Identification: B. bufo bufo, B. americanus, B. calamita, B. fowleri, B. gargarizans, B. marinus, (Erspamer, 1954); *B. viridis*, (Erspamer, 1959). *B. alvarius*, 30–40 μg per gram dried skin (Erspamer *et al.*, 1965).

N-Methyl-5-methoxytryptamine (XVIII). HCl, mp 166°–167°C; picrate, mp 220°–221°C (Wilkinson, 1958).
Identification: B. alvarius, 20–23 μg per gram dried skin (Erspamer *et al.*, 1965).

Bufotenine; *N,N*-dimethyl-5-hydroxytryptamine (XIX), mp 125°–126°C (Barlow and Khan, 1959; Iacobucci and Rúveda, 1964); 146°–147°C (H. Wieland *et al.*, 1934). Oxalate, mp 178°C (H. Wieland *et al.*, 1932). Monopicrate, mp 177.5°C. Dipicrate, mp 174°C (H. Wieland and Wieland, 1937; Hoshino and Shimodaira, 1935). Picrolonate, mp 120°–121°C (Deulofeu and Berinzaghi, 1946); 183°–184°C (Iacobucci and Rúveda, 1964). Flavianate, mp 130°–131°C (Jensen and Chen, 1932). UV spectrum; λ_{max} 225 mμ

Table II (*Continued*)

(loge 1.35), 280 (3.83) shoulder 303 (3.71) (ethanol). λ_{max} 277 (3.74), 296 (3.67) (0.1 *N* HCl). λ_{max} 218 (4.37), 376 (3.74), 323 (3.65) (0.1 *N* NaOH) (Stoll *et al.*, 1955).
Identification: B. americanus, B. calamita, B. fowleri (Erspamer, 1954). *B. viridis,* 630 μg per gram of fresh skin (Erspamer, 1959). *B. crucifer* (Pereira and de Oliveira, 1961).
Isolation: B. bufo bufo (Handovsky, 1920), 510 μg per animal (H. Wieland *et al.*, 1934); 47 μg per animal and 0.3% in dried secretion (males); 90 μg per animal and 0.33% in dried secretion (females) (H. Wieland and Behringer, 1941). Ch'an Su (Jensen and Chen, 1930). *B. arenarum* (Jensen and Chen, 1932); 5.1 mg per dried skin (H. Wieland *et al.*, 1934). *B. viridis* (Jensen and Chen, 1932) 0.06% in fresh skin (Erspamer, 1959). *B. paracnemis* (Deulofeu and Mendive, 1938). *B. chilensis, B. crucifer* (Deulofeu and Duprat, 1944). *B. formosus* (S. Ohno *et al.*, 1961). *B. alvarius,* 0.8–5 mg per gram dried glands, 0.33–2.15 mg per gram dried skin (Erspamer *et al.*, 1965).

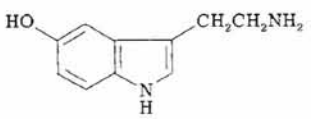
Bufoviridine, bufotenine *O*-sulfate (XX), mp 210°–212°C (Erspamer, 1959).
Identification: B. calamita (Erspamer, 1959).
Isolation: B. viridis (Erspamer, 1959).

O-Methylbufotenine; *N,N*-dimethyl-5-methoxytryptamine (XXI), mp 66°–67°C; picrate, mp 176°–177°C; methiodide, mp 183° (Hoshino and Shimodaira, 1936).
Identification: B. alvarius, 60–160 mg per gram of dried glands, 1.0–3.5 mg per gram dried skin (Erspamer *et al.*, 1965).

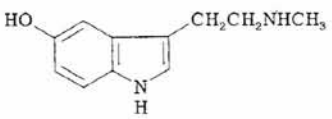
Bufotenidine; cinobufagin; *N,N,N*-trimethyl-5-hydroxytryptamine (XXII). Iodide, mp 216°–217°C (H. Wieland and Wieland, 1937). Oxalate, mp 96.5°C; picrate, mp 198°C, flavianate, mp 195°–200°C (H. Wieland *et al.*, 1934). Picronolate, mp 255°C (Deulofeu and Berinzaghi, 1946).
Identification: B. americanus, B. calamita, B. gargarizans, B. paracnemis, B. viridis (Erspamer, 1954).
Isolation: Ch'an Su (Chen *et al.*, 1931, H. Wieland *et al.*, 1932) *B. gargarizans, B. fowleri* (Jensen and Chen, 1932). *B. bufo bufo,* 170 μg per animal (H. Wieland *et al.*, 1934), 84 μg per animal and 0.53% in dried secretion (males), 170 μg per animal and 0.62% in dried secretion (females) (H. Wieland and Behringer, 1941). *B. formosus,* 2% in dried secretion (A. Ohno and Komatsu, 1957).
Other genera: isolation from *Xenopus laevis* (Jensen, 1935).

Bufothionine, dehydrobufotenine *O*-sulfate (XXIV). mp 250°C (H. Wieland and Vocke, 1930).
Isolation: B. formosus, 2 mg per dried skin (H. Wieland and Vocke, 1930). *B. arenarum,* 7.1 mg per dried skin (H. Wieland *et al.*, 1934). *B. chilensis, B. crucifer, B. paracnemis, B. spinulosus* (Deulofeu and Duprat, 1944).

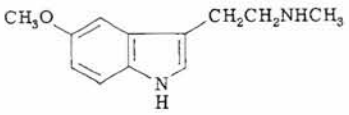
Dehydrobufotenine (XXIII), mp 218°C (H. Wieland and Wieland, 1937). HCl, mp 242°C (H. Wieland and Vocke, 1930). Picrate, mp 186°C (H. Wieland and Wieland, 1937). Picronolate, mp > 300°C (Deulofeu and Berinzaghi, 1946). Flavianate, mp 260°–265°C (H. Wieland *et al.*, 1934).
Identification: B. bufo bufo, B. americanus, B. calamita, B. fowleri, B. paracnemis, B. viridis (Erspamer, 1954).
Isolation: B. marinus (Jensen and Chen, 1932), 6 mg per animal (Märki *et al.*, 1961). *B. valliceps* (Jensen and Chen, 1932). *B. arenarum* (H. Wieland *et al.*, 1934). *B. regularis* (Jensen, 1935). Ch'an Su (Chen *et al.*, 1931). *B. chilensis, B. crucifer, B. paracnemis, B. spinulosus* (Deulofeu and Duprat, 1944).



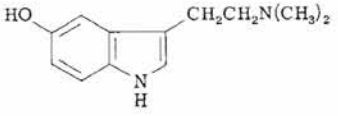
(XVI)



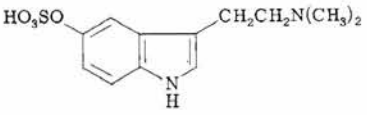
(XVII)



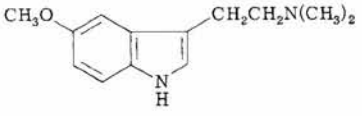
(XVIII)



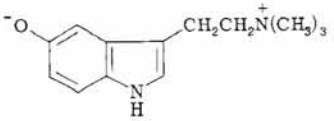
(XIX)



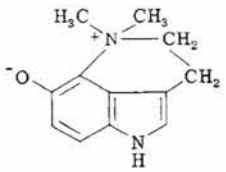
(XX)



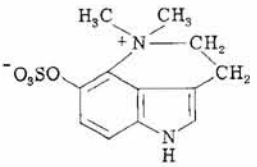
(XXI)



(XXII)



(XXIII)



(XXIV)

1. *5-Hydroxytryptamine (Serotonin, Enteramine, Thrombocytin)* (XVI)

The identification of 5-hydroxytryptamine in different animal tissues and its responsibility for several physiological activities was particularly the result of researches carried out by two groups of workers. In Italy, Erspamer and co-workers studied the substance responsible for the typical histochemical properties of the enterochromaffin cells, initially of the gastrointestinal mucosa and later of many other organs. In America, Rapport and Page were investigating the principle responsible for the vasoconstrictor properties of serum (for reviews on 5-hydroxytryptamine and related bases, see Erspamer 1954, 1961; Erspamer 1966; Page, 1954, 1958; Daly and Witkop, 1963; Lewis, 1964).

When both substances were isolated in pure form as salts or complexes (Rapport *et al.*, 1948; Erspamer and Asero, 1952) and their structure determined, they were found to be identical to 5-hydroxytryptamine (Rapport, 1949).

Afterwards, 5-hydroxytryptamine was found to have a widespread distribution in organs of many species of animals and to be of important significance, especially because of its relation to brain function and metabolism.

a. Chemistry. That the substance which determines the vasoconstrictor properties of serum was identical to 5-hydroxytryptamine was rightly proposed by Rapport (1949) and confirmed early by synthesis (Hamlin and Fischer, 1951; Speeter *et al.*, 1951; Erspamer and Asero, 1952; Asero *et al.*, 1952). Several syntheses of serotonin were developed afterwards (Speeter and Anthony, 1954; Harley-Mason and Jackson, 1954; Ek and Witkop, 1954; Young, 1958; Noland and Hovden, 1959; Kondo *et al.*, 1959; Suvorov and Murasheva, 1960).

b. Biosynthesis. The main steps in the biosynthesis of 5-hydroxytryptamine are indicated in Fig. 3.

There is a large amount of direct and indirect evidence of the capacity of microorganisms and higher animals for hydroxylating tryptophan (XXV) at carbon 5, with formation of 5-hydroxytryptophan (XXVI). L-Tryptophan labeled with ^{14}C has been transformed in patients suffering from carcinoidosis into labeled 5-hydroxy-L-tryptophan (XXVI) (Sjoerdsma *et al.*, 1957). The same hydroxylation has been observed *in vitro* by the action of particulate fractions of intestinal mucosa cells (rat, guinea pig) (Cooper and Melcer, 1961). Phenylalanine hydroxylase from the liver of rats is also capable of 5-hydroxylating tryptophan (Reuson *et al.*, 1962).

The 5-hydroxytryptophan is then decarboxylated enzymically with production of 5-hydroxytryptamine (XXVII) (Buzard and Nytch, 1957). Administration of 5-hydroxy-DL-tryptophan (XXVI) to rats and other higher

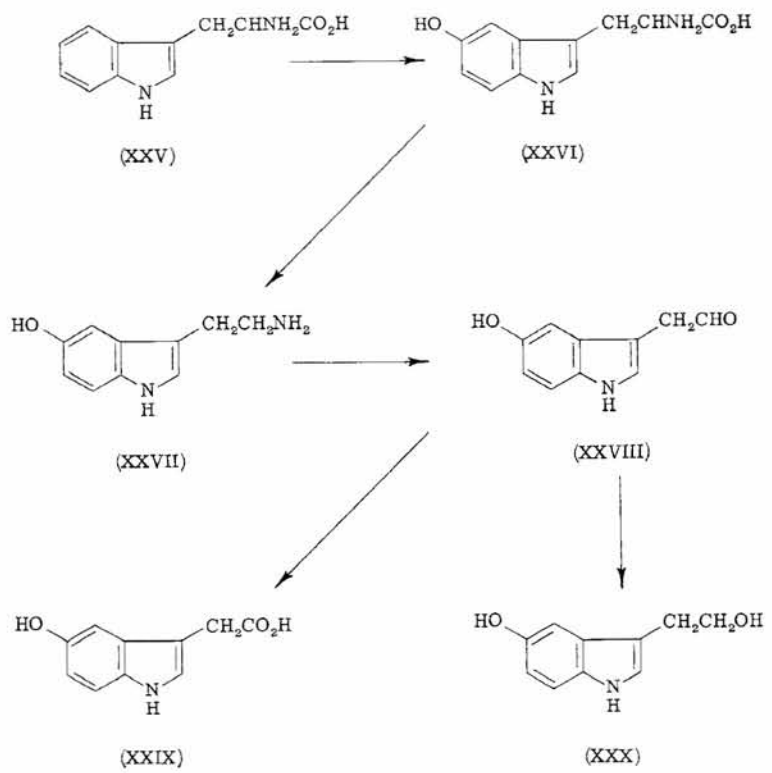


FIG. 3. Main pathways in the biogenesis and metabolism of 5-hydroxytryptamine.

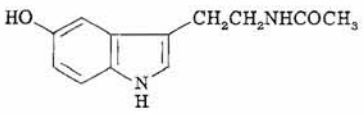
animals produces an increase in the amount of 5-hydroxytryptamine present in tissues and in the excretion of 5-hydroxyindoleacetic acid (XXIX) in the urine, both deriving from the L-isomer (Davidson *et al.*, 1957; Udenfriend *et al.*, 1957). If large amounts are administered, 5-hydroxytryptamine appears (rats) in the urine.

In the toad *B. marinus*, 5-hydroxylation of tryptophan also takes place, because after administration of labeled L-tryptophan (XXV) to the whole animals, active 5-hydroxy-L-tryptophan (XXVI) was isolated from the venom glands. Labeled dehydrobufotenine (XXIII) was also isolated (Udenfriend *et al.*, 1956).

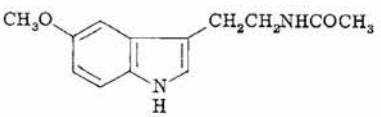
c. Metabolism. The products of 5-hydroxytryptamine metabolism vary with the species. Oxidation produces 5-hydroxyindoleacetic acid (XXIX), which is excreted in the urine, and was found to be an important metabolite in rats and rabbits.

It is partially conjugated with D-glucuronic acid, a small amount of

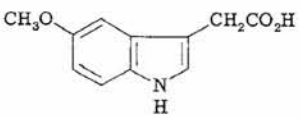
conjugation with sulfuric acid being also found (Erspamer and Bertaccini, 1962). An intermediate in the oxidation of 5-hydroxytryptamine (XVI) to 5-hydroxyindoleacetic acid (XXIX) is 5-hydroxyindoleacetaldehyde (XXVIII). Its reduction product, 5-hydroxytryptophol (XXX), appears to be one of the major metabolites found in the urine of rats, receiving 5-hydroxytryptophan (Kveder *et al.*, 1962). Some *N*-acetylation (XXXI) of 5-hydroxytryptamine has also been observed in rats, which is interesting, because of the structural relation of *N*-acetyl-5-hydroxytryptamine (XXXI) to melatonin (XXXII) the substance isolated from the pineal gland of cattle by Lerner *et al.* (1960), which is active on the melanocytes and produces paling of the frog, toad, and fish skins.



(XXXI)



(XXXII)



(XXXIII)

The formation of melatonin (XXXII) implies also the existence of a reaction of *O*-methylation of 5-hydroxyindoles and an enzyme catalyzing it has been found in the pineal body by Axelrod and Weissbach (1961). In this context the characterization in the skin of *B. alvarius* of 5-methoxyindoleacetic acid (XXXIII) is of interest (Erspamer *et al.*, 1965).

In the skin and venom of many species of toads and in the skin of other species of amphibians and in plants, compounds deriving from the *O*-methylation or/and *N*-methylation of 5-hydroxytryptamine have been isolated or characterized. They are considered as ulterior metabolic products of that amine and will be described individually in the following pages (see bufotenine, bufotenidine, dehydrobufotenine, and derivatives).

d. Distribution. 5-Hydroxytryptamine has a wide distribution in the animal kingdom. It has been found in the blood, gastrointestinal tract, nervous system, spleen, etc., of vertebrates. It is also present in the venom of certain reptiles, scorpions, and in the venomous apparatus of mollusks and coelenterates, the ganglia and nerve cords of several invertebrates.

Erspamer and Vialli (1951, 1952) investigated the presence of

5-hydroxytryptamine in the extracts from many amphibian skins by paper chromatography (Table II). The presence of 5-hydroxytryptamine in the venom of certain species of toads has been studied in particular for *B. marinus* (Udenfriend *et al.*, 1952), *B. bufo bufo* (Spandrio, 1961), *B. arenarum* (Frydman and Deulofeu, 1961), and *B. alvarius* (Erspamer *et al.*, 1965).

2. *N-Methyl-5-hydroxytryptamine* (XVII)

This amine was characterized for the first time by Erspamer and Vialli (1951, 1952) in the extracts from skins and parotoids from several species of *Bufo* by paper chromatography. It has not been found in other genera of amphibians.

3. *N-Methyl-5-methoxytryptamine* (XVIII)

In animals this base has been found only in the extracts of the skin of *B. alvarius* (Erspamer *et al.*, 1965). It has been isolated from several species of plants: *Phalaris arundinacea* (Wilkinson, 1958); *Piptadenia peregrina* (Legler and Tschesche, 1963; Iacobucci and Rúveda, 1964) and *Desmodium pulchellum* (Ghosal and Mukkerjee, 1965).

4. *N,N-Dimethyl-5-hydroxytryptamine* (*Bufotenine*) (XIX)

Bufotenine was not only the first indolic base isolated from toad venoms but also the first 5-hydroxytryptamine derivative isolated from organisms and synthesized. Its indolic structure was suspected by H. Wieland *et al.*, (1932) and by Jensen and Chen (1932). Further work settled the structure (H. Wieland *et al.*, 1934) and it was synthesized a year later by Hoshino and Shimidaira (1935). Recently Erspamer (1959) isolated from the secretion of *B. viridis* its sulfuric acid conjugate, which has been named bufoviridine (XX).

a. Chemistry. The interest in 5-hydroxytryptamine derivatives has produced in recent years a series of syntheses of bufotenine (Speeter and Anthony 1954; Harley-Mason and Jackson, 1954; Stoll *et al.*, 1955; Kondo *et al.*, 1959, 1960).

b. Distribution. In animals, with a few exceptions, bufotenine has been found only in the skin and secretion of species of the genus *Bufo* (toads, see Table II). It was not found by Erspamer and Vialli (1951) in the extracts of skins of other genera of amphibians, where 5-hydroxytryptamine was present.

Bufotenine and some of its derivatives have been found in plants. Some of the species containing them have been employed for smoking during tribal rites and no doubt the hallucinogenic properties of bufotenine played a part in their use. Stromberg (1954) was the first to isolate it from *Piptadenia peregrina* (Leguminoseae) (see also Alvares Pereira *et al.*, 1963); it was found in other species: *P. macrocarpa* (Fish *et al.*, 1955); *P. colubrina* (Pachter *et al.*, 1959); *P. excelsa* (Iacobucci and Rúveda, 1954), and *P. falcata* (Mennucci

Giesbrecht, 1960), in *Desmodium pulchellum* (Leguminosae) (Ghosal and Mukkerjee, 1964), and in *Phalaris tuberosa* (Gramineae) (Culvenor *et al.*, 1964). It was also isolated or identified in several species of *Amanita* although it is absent in others. (T. Wieland and Motzel, 1953; Tyler, 1961). The *N*-oxide of bufotenine has been isolated or identified in *P. peregrina*, *P. macrocarpa* (Fish *et al.*, 1955), *P. excelsa* (Iacobucci and Rúveda, 1964), and *D. pulchellum* (Ghosal and Mukkerjee, 1964).

5. *N,N-Dimethyl-5-hydroxytryptamine-O-sulfate (Bufoviridine)* (XX)

From the many species of amphibians investigated by Erspamer and Vialli (1951, 1952) bufoviridine (XVIII) was detected only in *B. viridis* and *B. calamita*. Later it was isolated from *B. viridis* by Erspamer (1959). On acid hydrolysis, bufotenine (XVII) and sulfuric acid were produced, showing that bufoviridine was the sulfuric ester of the base.

The formation of bufoviridine is an indication of the capacity for the sulfoconjugation of 5-hydroxyindole derivatives which is found in toads. It parallels the sulfoconjugation of dehydrobufotenine to bufothionine, which will be considered later.

6. *N,N-Dimethyl-5-methoxytryptamine (O-Methylbufotenine)* (XXI)

O-methylbufotenine has been isolated for the first time from an animal source from the skin and glands of *B. alvarius* (Erspamer *et al.*, 1965). It was already known to be present in some plants: *Dictyloma incanescens* (*Rutaceae*) (Pachter *et al.*, 1959); *P. peregrina* (Legler and Tschesche, 1963) and *Phalaris tuberosa* (Culvenor *et al.*, 1964). It is also present in *D. pulchellum* which also contains its *N*-oxide (Ghosal and Mukkerjee, 1964, 1965).

7. *N,N,N-Trimethyl-5-hydroxytryptamine (Bufotenidine, Cinobufagine)* (XXII)

Cinobufagine was the name given to a base isolated as flavianate from Ch'an Su by Jensen and Chen (1930). H. Wieland *et al.* (1932), who isolated it from the same source and found that it was also present in the secretion of *B. vulgaris*, named the base bufotenidine. He showed that bufotenidine resulted from the quaternization of bufotenine, on treatment with methyl iodide. It has been found in the skin and secretion of many species of toads investigated (Table II), but not in all of them, in spite of the fact that they contain bufotenine, which can be considered a logical precursor. It is present in the skin of *Xenopus laevis*.

a. Biosynthesis of the N-Methyl-5-hydroxytryptamines. The bufotenine (XIX) and bufotenidine (XXII) found on toad venoms are considered to be derived from 5-hydroxytryptamine by *N*-methylation. *N*-Methylation of tryptamines does not seem to be important in mammals and even in many other lower species of animals where 5-hydroxytryptamine has been detected and where only in very exceptional cases its *N*-methylated derivatives have

been found. The data collected by Erspamer (1954, 1961) on the occurrence of indolethylamines in the amphibian skin shows this clearly.

Enzymes which can catalyze the *N*-methylation of indolethylamines have been described. Axelrod (1961, 1962) found in rabbit lungs an unspecific *N*-methyl transferase which methylates 5-hydroxytryptamine with production of *N*-methyl-5-hydroxytryptamine and also of this last compound, with production of bufotenine (XIX). The unspecific *N*-methyl transferase found in phenylethylamines can also methylate tryptamine derivatives. It seems to be similar although not identical to the enzyme present in the lungs.

The isolation from animals and plants of 5-methoxytryptamines in different stages of *N*-methylation is indicative that *O*-methylation is an enzymic reaction common to certain species of the two kingdoms. In this connection the isolation from the skin and glands of *B. alvarius* of several 5-methoxyindole derivatives (Erspamer *et al.*, 1965) must be noted.

There is no information available to decide if *N*-methylation takes precedence over *O*-methylation or vice versa.

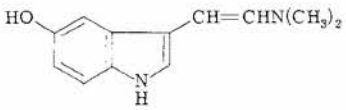
b. Metabolism of the N-Methyl-5-hydroxytryptamines. We do not have any experimental information on the ulterior metabolism of bufotenine (XIX) and bufotenidine (XXII) in the toad. The presence of bufoviridine (XX) in two species shows that in some cases *O*-sulfonation can take place.

Some studies have been made in man and higher animals on the fate of administered bufotenine. Compared to 5-hydroxytryptamine, it is slowly metabolized by monoamine oxidase with the result that in rats, only a small percent of the administered amount is transformed and excreted as 5-hydroxyindoleacetic acid (XXX). A much larger proportion is found in the urine unchanged or conjugated with glucuronic acid (Gessner *et al.*, 1960).

It is interesting that 5-methoxyindoleacetic acid (XXXIII) has been found in the skin and glands of *B. alvarius* which also contains *O*-methylbufotenine and *N*-methyl-5-methoxytryptamine (Erspamer *et al.*, 1965).

8. *Dehydrobufotenine (XXIII) and Bufothionine (XXIV)*

Bufothionine (XXIV) was first isolated by H. Wieland and Vocke (1930) from *B. gama*. By acid hydrolysis, sulfuric acid and a basic substance which was named dehydrobufotenine (XXIII) were produced. The last compound was later found identical to a base, isolated as flavianate from several species of toad (Jensen and Chen, 1932; H. Wieland *et al.*, 1934). Bufothionine was obviously the sulfuric ester of the base.



(XXXIV)

a. Chemistry. On the basis of its transformation to bufotenine by hydrogenation, dehydrobufotenine was assigned the structure (XXXIV) by H. Wieland and Wieland (1937). This structure was put in doubt by Witkop (1956) who noticed that its ultraviolet spectrum was closely similar to that of 5-hydroxyindole. In 1961, two groups of workers (Märki *et al.*, 1961; Robinson *et al.*, 1961), proposed, on the basis of the nuclear magnetic resonance spectrum, a structure (XXIII) for dehydrobufotenine, from which is derived the structure (XXIV) for bufothionine.

Structure (XXIII) explains all the chemical reactions described for dehydrobufotenine and also the physical properties of the compound. Dehydrobufotenine as the free base or as the sulfoconjugate, bufothionine, has been found in many species of toads (Table II). It has not been found outside the genus *Bufo*, with the exception of the amphibian *Acris crepitans* in whose skin extracts Erspamer and Vialli (1952) detected bufothionine as the only indolic component, by paper chromatography.

b. Metabolism. That 5-hydroxytryptophan is a precursor of dehydrobufotenine was shown by Udenfriend *et al.* (1956) who found that after the administration of radioactive DL-tryptophan to *B. marinus*, labeled dehydrobufotenine could be isolated from the venom glands of the toad.

Although we have no evidence, it is plausible to postulate that dehydrobufotenine is formed from bufotenine by nucleophilic attack of the amino nitrogen atom on the 4-carbon atom of the indole nucleus, under enzymic catalysis. The ultimate fate of the base, except that it can be sulfoconjugated to form bufothionine (XXIV), is not known.

V. ANALYTICAL METHODS

The investigation of the bases present in extracts of toad skins, glands, or secretions can be done by paper chromatography. A table of R_f for several amines, in four different solvent systems, has been published by Reio (1960). Thin layer chromatography has been applied to dopamine derivatives by Kuehl *et al.* (1964).

Erspamer and Vialli (1951, 1952) and Erspamer (1959) have described several solvent systems which can be employed in paper chromatography for the identification of indolic bases. Chromatographic methods combined with the use of radioactive derivatives have been employed by Udenfriend *et al.* (1952) and by Märki *et al.* (1962).

Column chromatography has been used for the isolation of some bases, for example bufoviridine (Erspamer, 1959). Ion exchange columns have been used with success by Märki *et al.* (1961) for the isolation of dehydrobufotenine.

A gas chromatographic method for the separation and identification of

the indolic bases present in plant material has been reported by Holmstedt *et al.* (1964) and has been successfully applied to plant products (Holmstedt, 1965).

By applying mass spectrometry to the fractions separated by gas chromatography, Holmstedt (1967) has developed a powerful method of identification, which he has applied with success to South American snuffs and plants.

Colored reactions for bufotenine and bufotenidine have been described by Hamet and Lelogeais (1954). The application and study of the colors given by a modified Keller reaction to indolic bases, including bufotenine, has been done by Rieder and Böhner (1959).

REFERENCES

Abel, J. J., and Macht, D. I. (1911–1912). *J. Pharmacol. Exptl. Therap.* **3**, 319.
Alvarez Pereira, N., Marins, I. C., and Moussatché, H. (1963). *Rev. Brazil. Biol.* **23**, 211.
Asero, B., Coló, V., Erspamer, V., and Vercellone, A. (1952). *Ann. Chem.* **576**, 69.
Axelrod, J. (1959). *Physiol. Rev.* **39**, 751.
Axelrod, J. (1961). *Science* **134**, 343.
Axelrod, J. (1962). *J. Pharmacol. Exptl. Therap.* **138**, 28.
Axelrod, J. (1963). *Science* **140**, 499.
Axelrod, J., and Tomchick, R. (1958). *J. Biol. Chem.* **233**, 702.
Axelrod, J., and Weissbach, H. (1961). *J. Biol. Chem.* **236**, 211.
Axelrod, J., Inscoe, J. K., Senoh, S., and Witkop, B. (1958a). *Biochim. Biophys. Acta* **27**, 210.
Axelrod, J., Senoh, S., and Witkop, B. (1958b). *J. Biol. Chem.* **233**, 696.
Axelrod, J., Wurtman, R. J., and Winget, C. M. (1964). *Nature* **201**, 1134.
Barger, G., and Ewins, L. (1910). *J. Chem. Soc.* p. 2257.
Barlow, R. B., and Khan, I. (1959). *Brit. J. Pharmacol.* **14**, 265.
Bretschneider, H. (1947). *Monatsh. Chem.* **78**, 82.
Bridgers, W. F., and Kaufman, S. (1962). *J. Biol. Chem.* **237**, 526.
Buzard, J. A., and Nytch, P. D. (1957). *J. Biol. Chem.* **227**, 225.
Chen, K. K., and Chen, A. L. (1933). *J. Pharmacol. Exptl. Therap.* **49**, 503.
Chen, K. K., Jensen, H., and Chen, A. L. (1931). *J. Pharmacol. Exptl. Therap.* **43**, 13.
Cooper, S. R., and Melcer, I. (1961). *J. Pharmacol. Exptl. Therap.* **132**, 265.
Culvenor, C. C. J., Dal Bon, R., and Smith, L. W. (1964). *Australian J. Chem.* **17**, 1301.
Daly, J. W., and Witkop, B. (1963). *Angew. Chem.* **75**, 552; *Angew. Chem. Intern. Ed. Engl.* **2**, 421 (1963).
Davidson, J. D., Loom, S., and Udenfriend, S. (1957). *J. Clin. Invest.* **36**, 1954.
Deulofeu, V. (1935). *Z. Physiol. Chem.* **237**, 171.
Deulofeu, V. (1948). *Fortschr. Chem. Org. Naturstoffe* **5**, 241.
Deulofeu, V., and Berinzaghi, B. (1946). *J. Am. Chem. Soc.* **68**, 1665.
Deulofeu, V., and Duprat, E. (1944). *J. Biol. Chem.* **153**, 459.
Deulofeu, V., and Mendive, J. R. (1938). *Ann. Chem.* **534**, 288.
Ek, A., and Witkop, B. (1954). *J. Am. Chem. Soc.* **76**, 5579.
Erspamer, V. (1954). *Pharmacol. Rev.* **6**, 425.
Erspamer, V. (1959). *Biochem. Pharmacol.* **2**, 270.

Erspamer, V. (1961). *Progr. Drug Res.* **3**, 151.
Erspamer, V. (1966). "5-Hydroxytryptamine and Related Indolealkylamines," Vol. 19, Heffter-Heubner *Handbuch of Experimental Pharmacology*. Springer, Berlin.
Erspamer, V., and Asero, B. (1952). *Nature* **169**, 800; *J. Biol. Chem.* **200**, 311 (1900).
Erspamer, V., and Bertaccini, G. (1962). *Arch. Intern. Pharmacodyn.* **137**, 6.
Erspamer, V., and Vialli, M. (1951). *Nature* **167**, 1033.
Erspamer, V., and Vialli, M. (1952). *Ric. Sci.* Suppl. **22**, 1421.
Erspamer, V., Vitali, T., Roseghini, M., and Cei, J. M. (1965). *Experientia* **21**, 504.
Fischer, P., and Lecomte, J. (1950). *Arch. Intern. Physiol.* **57**, 277.
Fish, M. S., Johnson, N. M., and Horning, E. C. (1955). *J. Am. Chem. Soc.* **77**, 5892.
Frydman, B., and Deulofeu, V. (1961). *Experientia* **17**, 545.
Gessner, P. K., Khairallah, P. A., McIsaac, W. M., and Page, I. H. (1960). *J. Pharmacol. Exptl. Therap.* **130**, 126.
Ghosal, S., and Mukkerjee, B. (1964). *Chem. Ind. (London)* p. 1800.
Ghosal, S., and Mukkerjee, B. (1965). *Chem. Ind. (London)* p. 793.
Hamet, R., and Lelogeais, P. (1954). *Bull. Soc. Chim. Biol.* **36**, 933.
Hamlin, K. E., and Fischer, F. E. (1951). *J. Am. Chem. Soc.* **73**, 5007.
Handovsky, H. (1920). *Arch. Exptl. Pathol. Pharmakol.* **86**, 138.
Harley-Mason, J., and Jackson, A. H. (1954). *J. Chem. Soc.* p. 1165.
Hertting, G., and La Brosse, E. H. (1962). *J. Biol. Chem.* **237**. 2291.
Holmstedt, B. (1965). *Arch. Intern. Pharmacodyn.* **156**, 285.
Holmstedt, B. (1967). *In* "Ethnopharmacologic Search for Psychoactive Drugs" (D. H. Efron, B. Holmstedt, and N. S. Kline, eds.). Public Health Service, Washington.
Holmstedt, B., Vandenheuvel, W. J. A., Gardiner, W. L., and Horning, E. C. (1964). *Anal. Biochem.* **8**, 151
Hoshino, T., and Shimodaira, K. (1935). *Ann. Chem.* **520**, 19.
Hoshino, T., and Shimodaira, K. (1936). *Bull. Chem. Soc. Japan* **11**, 221; *Chem. Abstr.* **30**, 5982 (1936).
Iacobucci, G. A., and Rúveda, E. A. (1964). *Phytochemistry* **3**, 465.
Jaminet, F. (1959). *Farmaco. (Pavia), Ed. Sci.* **14**, 120.
Jensen, H. (1935). *J. Am. Chem. Soc.* **57**, 1765.
Jensen, H., and Chen, K. K. (1929). *J. Biol. Chem.* **82**, 397.
Jensen, H., and Chen, K. K. (1930). *J. Biol. Chem.* **87**, 741.
Jensen, H., and Chen, K. K. (1932). *Chem. Ber.* **65**, 1310.
Kaiser, E., and Michl, H. (1958). "Die Biochemie der tierischen Gifte." Franz Deuticke, Vienna.
Kondo, H., Kataoka, H., Hayashi, V., and Dodo, T. (1959). *Ann. Rep. Itsuu Lab.* **10**, 1; *Chem. Abstr.* **54**, 492 (1960).
Kondo, H., Kataoka, H., and Dodo, T. (1960). *Ann. Rep. Itsuu Lab.* **11**, 53; *Chem. Abstr.* **55**, 17619 (1961).
Kveder, S., Iskrič, V. S., and Keglevič, D. (1962). *Biochem. J.* **85**, 447.
Kuehl, F. A., Hichens, M., Ormond, R. E., Meisinger, M. A. P., Gale, P. H., Cirillo, V. J., and Brink, N. G. (1964). *Nature* **203**, 154.
Lasagna, L. (1951). *Proc. Soc. Exptl. Biol. Med.* **78**, 876.
Lee, H. M., and Chen, K. K. (1951). *J. Pharmacol. Exptl. Therap.* **102**, 286.
Legler, G., and Tschesche, R. (1963). *Naturwissenschaften* **50**, 94.
Lerner, A. B., Case, J. D., and Takahashi, Y. (1960). *J. Biol. Chem.* **235**, 1992.
Lewis, G. P. (1964). *In* "The Hormones" (G. Pincus, K. V. Thimann, and E. B. Astwood, eds.), Vol. 4, pp. 387–402. Academic Press, New York.
Märki, F., and Witkop, B. (1961). Quoted by Märki *et al.* (1961).

Märki, F., Robertson, A. V., and Witkop, B. (1961). *J. Am. Chem. Soc.* **83**, 3341.
Märki, F., Axelrod, J., and Witkop, B. (1962). *Biochim. Biophys. Acta* **58**, 367.
Mennucci Giesbrecht, A. (1960). *Anais Assoc. Brasil. Quim.* **19**, 117.
Noland, W. E., and Hovden, R. A. (1959). *J. Org. Chem.* **24**, 894.
Ohno, S., and Komatsu, M. (1957). *Ann. Rep. Itsuu Lab.* **8**, 31; (*Chem. Abstr.* **51**, 14984 (1957).
Ohno, S., Ohmoto, T., and Komatsu, M. (1961). *Yakugaku Zasshi* **81**, 1339; (*Chem. Abstr.* **56**, 7391 (1962).
Östlund, E. (1954). *Acta Physiol. Scand.* **31**, Suppl. 112, 167.
Pachter, I. J., Zacharius, D. E., and Ribeiro, O. (1959). *J. Org. Chem.* **24**, 1285.
Page, I. H. (1954). *Physiol. Rev.* **34**, 563.
Page, I. H. (1958). *Physiol. Rev.* **38**, 277.
Pereira, N. A., and de Oliveira, D. S. (1961). *Rev. Brasil. Farm.* **42**, 13; (*Chem. Abstr.* **56**, 6486 (1962).
Phisalix, C., and Bertrand, G. (1893). *Compt. Rend.* **116**, 1080.
Phisalix, C., and Bertrand, G. (1902). *Compt. Rend.* **135**, 46.
Piccinelli, D. (1955). *Boll. Soc. Eustachiana Ist. Sci. Univ. Camerino* **44**, 105; (*Chem. Abstr.* **53**, 8327 (1959).
Pratesi, P., La Manna, A., Campiglio, A., and Ghislandi, V. (1958). *J. Chem. Soc.* p. 2069.
Pratesi, P., La Manna, A., Campiglio, A., and Ghislandi, V. (1959). *J. Chem. Soc.* p. 4062.
Rapport, M. M. (1949). *J. Biol. Chem.* **180**, 961.
Rapport, M. M., Green, A. A., and Page, I. H. (1948). *Science* **108**, 329; *J. Biol. Chem.* **176**, 1243 (1948).
Reio, L. (1960). *J. Chromatog.* **4**, 458.
Reuson, J., Weissbach, H., and Udenfriend, S. (1962). *J. Biol. Chem.* **237**, 2261.
Rieder, H. P., and Böhner, M. (1959). *Helv. Chim. Acta.* **42**, 1793.
Robinson, B., Smith, G. F., Jackson, A. H., Shaw, D., Frydman, B., and Deulofeu, V. (1961). *Proc. Chem. Soc.* p. 310.
Rosen, L., and Goodall, M. (1962). *Proc. Soc. Exptl. Biol. Med.* **110**, 767.
Sjoerdsma, A., Weissbach, H., and Udenfriend, S. (1957). *Am. J. Med.* **23**, 5.
Slotta, K. H., Valle, J. C., and Neisser, K. (1937). *Mem. Inst. Butantan* (*São Paulo*) **11**, 101.
Spandrio, L. (1961). *Arch. Sci. Biol.* (*Bologna*) **45**, 337.
Speeter, M. E., and Anthony, W. C. (1954). *J. Am. Chem. Soc.* **76**, 6208.
Speeter, M. E., Heinzelmann, R. V., and Weisblat, D. I. (1951). *J. Am. Chem. Soc.* **73**, 5514.
Stoll, A., Troxler, F., Peyer, J., and Hofmann, A. (1955). *Helv. Chim. Acta* **38**, 1452.
Stromberg, V. L. (1954). *J. Am. Chem. Soc.* **76**, 1707.
Suvorov, N. N., and Murasheva, V. S. (1960). *Zh. Obshch. Khim.* **30**, 3112; (*Chem. Abstr.* **55**, 17620 (1961).
Tschesche, R. (1945). *Fortschr. Chem. Org. Naturstoffe* **4**, 1.
Tullar, B. F. (1948). *J. Am. Chem. Soc.* **70**, 2067.
Tyler, V. E. (1961). *Lloydia* **24**, 71.
Udenfriend, S., Clark, C. T., and Titus, E. (1952). *Experientia* **8**, 379.
Udenfriend, S., Titus, E., Weissbach, H., and Peterson, R. F. (1956). *J. Biol. Chem.* **219**, 335.
Udenfriend, S., Weissbach, H., and Bogdanski, D. F. (1957). *J. Biol. Chem.* **224**, 803.
Udenfriend, S., Lovenberg, W., and Sjoerdsma, A. (1959). *Arch. Biochem. Biophys.* **85**, 487.
van de Veerdonk, F. C. G., Huismans, J. W., and Addink, A. D. F. (1961). *Z. Vergleich, Physiol.* p. 22, **44**, 323.
von Euler, U. S. (1956). "Noradrenaline: Chemistry, Physiology, Pharmacology and Clinical Aspects." Thomas, Springfield, Illinois.

Waalkes, T., Sjoerdsma, A., Creveling, C. R., Weissbach, H., and Udenfriend, S. (1958). *Science* **127**, 648.
Wasser, E., and Sommer, H. (1928). *Helv. Chim. Acta.* **6**, 54.
Weiner, N. (1964). *In* "The Hormones" (G. Pincus, K. V. Thimann, and E. B. Astwood, eds.), Vol. 4, pp. 403–479. Academic Press, New York.
Wieland, H., and Behringer, H. (1941). *Ann. Chem.* **549**, 209.
Wieland, H., and Vocke, F. (1930). *Ann. Chem.* **481**, 213.
Wieland, H., and Wieland, T. (1937). *Ann. Chem.* **528**, 234.
Wieland, H., Hesse, G., and Mittasch, H. (1932). *Chem. Ber.* **64**, 2099.
Wieland, H., Konz, W., and Mittasch, H. (1934). *Ann. Chem.* **513**, 1.
Wieland, T., and Motzel, W. (1953). *Ann. Chem.* **581**, 10.
Wilkinson, S. (1958). *J. Chem. Soc.* p. 2079.
Witkop, B. (1956). *J. Am. Chem. Soc.* **78**, 2873.
Young, E. H. P. (1958). *J. Chem. Soc.* p. 3493.