unhydrous conditions, novel migrations of acetoxy groups were observed.

Four types of rearrangements can now be distinguished in the p-toluquinol acetate series:

(1) An *external addition of acetate anion* to the cation V*c* (arising *via* V*a* ⟷ V*b*) by the action of acetic anhydride in a *sulfuric acid-catalyzed* THIELE *reaction*. The product is (starting with V) diacetyl cresorcinol (XIII, liquid, yield about 70%), hydrolyzed by base to cresorcinol, m. p. 106–107°, identified by analysis, mixed melting point, and IR-spectrum.

(2) An *internal migration of the acetoxy group* by the action of boron trifluoride in ether involving a cyclic carbonium intermediate V*d* reminiscent of similar intermediates in replacement reactions in which complex neighboring groups participate[1]. The reaction product in this case is the new *monoacetyl cresorcinol* (XII, yield 70%), m. p. 102–104°, hydrolyzed to cresorcinol.

(3) Hydrolysis of the p-quinol acetate in aqueous *acidic* solution followed by *migration of the alkyl group* (V*e*) leading to toluhydroquinone (VIII) or to homogentisic acid (IX).

(4) Hydrolysis of the p-quinol acetate in aqueous *alkaline* medium followed by a *benzilic acid type of rearrangement* (VII*a* → VII*b*) leading again to derivatives of hydroquinone (VIII, IX).

When the o-quinone *ortho*-acetate (II) was dissolved in acetic anhydride in the presence of catalytic amounts of sulfuric acid at room temperature an exothermic THIELE reaction of an unusual kind occurred yielding the *triacetyl pyrrogallol* derivative IX, m. p. 101·5–102·5°. One of the possible routes in this, as we believe, combined *intra-* and *intermolecular acetylation process* is pictured in the hypothetical intermediates II*a* → II*e*. IV, on acid hydrolysis, furnished 3,4,5-trihydroxytoluene (m. p. 126–7°) which, after methylation to the liquid 3,4,5-trimethoxytoluene, gave, on oxidation with potassium permanganate in acetone, trimethylgallic acid (m. p. 157°) identified by analysis, mixed melting points and infrared spectra.

*To summarize:* p-quinol acetates can rearrange to hydroquinone as well as resorcinol derivatives, o-quinoid compounds of type II to pyrrogallol derivatives, whereby no change in the state of oxidation or reduction occurs. The formation of the o-quinoid compound II from I with lead tetraacetate may not necessarily go through the catechol state[2], a consideration which is significant for enzymatic reactions of a similar kind, such as the transformation of 3-hydroxyanthranilic acid to niacin. There 3,4-dihydroxyanthranilic acid, contrary to previous claims[3] is *not* the intermediate[4], but rather the o-quinoid compound (or an open analog[5]).

The quinols derived from p-cresol and hydroxyphenylacetate were not metabolized by the enzyme preparation from rat liver[6] as Dr. LA DU[7] found out. We comment on the negative result of such a test in the same way as FROMHERZ and HERMANNS[1] did forty years ago. Our results on the preparation and rearrangement of quinols with varying side chains derived from phenols I [$R_1$ = $C_6H_2COCH_3$; $CH_2$–CH(NHAc)COOR, etc.] will be reported elsewhere. That suitable negative centers of side chains, such as ethanamine, are capable of adding internally to the o-quinoid and p-quinoid systems II and V is known from previous work and considerations[2]. The loss of water from an intermediate such as X would lead to a system XI present in *β*-erythroidine[3] and, presumably in a slightly modified form, also in gliotoxin[4]. Model experiments in this direction are in progress.

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*Zusammenfassung*

Der Dualismus der o- und p-Hydroxylierung, der beim Tyrosin *in vivo* beobachtet wird, lässt sich *in vitro* mit Bleitetraazetat nach WESSELY an geeigneten p-substituierten Phenolen, wie zum Beispiel p-Oxyphenylessigsäureester, demonstrieren. Die o- und p-Azetoxylierung führt zu Azetaten o- und p-chinoider Verbindungen, die eine Fülle neuartiger säuren- und basenkatalysierter *intra-* und *inter*molekularer Umlagerungen zu Derivaten des Resorzins, Hydrochinons und Pyrrogallols zeigen.

[1] S. WINSTEIN, L. GOODMAN and R. BOSCHAN, J. Amer. Chem. Soc. *72*, 4669 (1950); XII intern. Congr. of Pure and Applied Chemistry, Abstracts Org. Chem., 436, September 1951, New York. Cf. S. WINSTEIN and R. E. BUCKLES, J. Amer. Chem. Soc. *65*, 613 (1943).

[2] Cf. J. N. SMITH, Biochem. Soc. Symposia, *5* 15 (1950).

[3] K. MAKINO, F. ITOH, and K. NISHI, Nature *167*, 115 (1951).

[4] L. M. HENDERSON, H. N. HILL, R. E. KOSKI, and I. M. WEINSTOCK, Proc. Soc. Exp. Biol. Med. *78*, 441 (1951).

[5] A. H. BOKMAN and B. S. SCHWEIGERT, Arch. Biochem. Biophys. *33*, 270 (1951). – Cf. A. BUTENANDT and H. G. SCHLOSSBERGER, Ber. dtsch. chem. Ges. *85*, 565 (1952).

[6] B. N. LA DU, Jr., and D. M. GREENBERG, J. Biol. Chem. *190*, 245 (1951).

[7] We are indebted to Dr. LA DU for these tests. Assaying of further quinols is in progress.

[1] K. FROMHERZ and L. HERMANNS, Z. Physiol. Chem. *91*, 213 (1914).

[2] *D.* RAPER, Biochem. J. *20*, 735 (1926); *21*, 89 (1927). – A. B. LERNER and T. B. FITZPATRICK, Physiological Review *30*, 191 (1950). – Cf. R. ROBINSON, Chemistry and Industry 358 (*1952*).

[3] V. PRELOG *et al.*, Helv. chim. Acta *34*, 1601, 1969 (1951). – V. BOEKELHEIDE, M. F. GRUNDON, and J. WEINSTOCK, J. Amer. Chem. Soc. *74*, 1866 (1952); A. C. S. Meeting, Atlantic City, Sept. 14–19, 1952, Abstracts, 12 M.

[4] Unpublished results on the hydrogenation of gliotoxin by Dr. J. D. DUTCHER (as well as spectrophotometric observations) make the assumption of an aromatic ring in gliotoxin untenable. We are grateful to Dr. DUTCHER for the communication of his results as well as for stimulating discussions.

## The Presence of 5-Hydroxytryptamine in the Venom of *Bufo marinus*

Serotonin, the vasoconstrictor substance in mammalian serum, has recently been identified by RAPPORT, GREEN, and PAGE[1] as 5-hydroxytryptamine. RAND and REID[2] have also shown that thrombocytin, the hemostatic agent in platelets, is probably 5-hydroxytryptamine. ERSPAMER and OTTOLENGHI[3] found that enteramine, which serves as a local hormone in the gut, is apparently 5-hydroxytryptamine. They have also found this substance in many invertebrate sources.

JENSEN and CHEN[4] and WIELAND[5], in 1934, established that various N-methyl derivatives of 5-hydroxytryptamine which have considerable pressor activity were present in large amount in toad venom. 5-Hydroxy-

[1] M. M. RAPPORT, A. A. GREEN, and I. H. PAGE, J. Biol. Chem. *176*, 1243 (1948).

[2] M. RAND and G. REID, Nature *168*, 385 (1951).

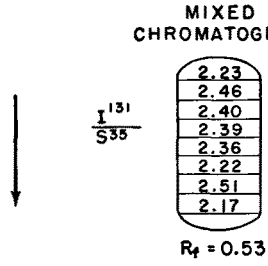
[3] V. ERSPAMER and A. OTTOLENGHI, Exper. *8*, 31 (1952).

[4] H. JENSEN and K. K. CHEN, J. Biol. Chem. *116*, 87 (1936).

[5] H. WIELAND, Ann. Chem. *513*, 1 (1934).

tryptamine was not reported by these authors, but one would expect it to be a precursor of the N-methylated derivatives. Isotopic and chromatographic techniques have måde it possible to demonstrate the presence of 5-hydroxytryptamine to the extent of about 0·1 per cent of the dry weight of *Bufo marinus* venom.

Approximately 2 g of dried venom obtained from 6 toads[1] were ground in a Potter glass homogenizer with a total of 400 ml of 0·02 N HCl in 75 per cent ethanol. The extract was evaporated *in vacuo* at 45° to about 80 ml and poured while still warm into 350 ml of 0·01 N HCl. After standing several hours to permit complete precipitation, the solids were removed by centrifugation and washed with 30–40 ml of 0·01 N HCl. The combined acid solution and washings were extracted twice with equal volumes of n-butanol and the butanol discarded.




Chromatogram of a mixture of $S^{35}$-labeled pipsyl derivative obtained from *Bufo marinus* venom and the $I^{131}$-labeled pipsyl derivative of an authentic sample of 5-hydroxytryptamine.

After cooling to 10–15°, the aqueous phase was adjusted with dilute NaOH to pH 10 and extracted three times with equal volumes of n-butanol. To the combined butanol extracts (about 1 l) was added 1 l of heptane. Water from the butanol layer separated out at this step. Three milliliters of 6 N HCl were added and the mixture shaken in a separatory funnel. The acid layer was withdrawn and the organic phase was re-extracted with 60 ml of water. The combined aqueous extracts were evaporated, under nitrogen, to approximately 10 ml, washed with an equal volume of n-butanol, and further evaporated to approximately 1 ml. The entire solution was deposited (as a line) across the tops of 2 sheets of WHATMAN No. 3 filter paper, and one dimensional chromatograms were developed using a mixture of butanol, propionic acid, and water in the proportions 5:2:3. The band of 5-hydroxytryptamine ($R_f$ about 0·25) was located by means of its characteristic pink fluorescence in ultraviolet light. This fluorescent band was cut from each sheet and eluted with 0·1 N HCl. The eluate was adjusted to a pH of 8·95 and a volume of 8 ml and subjected to 24 transfer counter-current distribution[2] between n-butanol and 0·1 *M* borate buffer, pH 8·95. In this system, pure 5-hydroxytryptamine has a partition ratio of about 1. Tubes 8 to 16 which contained the bulk of material possessing spectral characteristics similar to 5-hydroxytryptamine were pooled and concentrated to about 2 ml. A half milliliter portion containing about 100 $\mu$g of apparent 5-hydroxytryptamine was treated with $S^{35}$ labeled p-iodophenylsulfonylchloride (pipsyl chloride) to yield the corresponding derivative. An authentic sample of 5-hydroxytryptamine[3] was treated with $I^{131}$ labeled pipsyl chloride forming the corresponding $I^{131}$ labeled derivative. The $S^{35}$ labeled material obtained from the toad venom and the $I^{131}$ labeled derivative of pure 5-hydroxytryptamine exhibited identical $R_f$ values on paper chromatograms and a mixture of the two yielded a single spot[1]. Homogeneity was further established by demonstrating that $I^{131}/S^{35}$ ratios in consecutive transverse segments of the spot on the mixed chromatogram were constant throughout (see Figure)[2].

The finding of 5-hydroxytryptamine in the toad increases the number of animal species in which this material has now been found and further suggests its general physiological importance. Its presence in the toad gland in relatively large amounts should make the toad a useful experimental animal in which to study its biosynthesis. The presence of the methylated derivatives suggests that methylation may be a pathway for metabolism of 5-hydroxytryptamine in other animals. Until now, the methylated derivatives of 5-hydroxytryptamine have been considered peculiar to the toad; they may prove to be of more widespread physiological importance.

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*Zusammenfassung*

5-Hydroxytryptamin (Serotonin, Enteramin, Thrombocytin), das in neuerer Zeit aus Blut oder Gewebe verschiedener Tiere isoliert wurde, konnte nun auch unter Verwendung von Isotopentechnik und Papierchromatographie als unmethylierter Vorläufer der schon bekannten Krötenbasen im Giftextrakt von *Bufo marinus* im Ausmasse von 0,1 % (bezogen auf trockenen Giftextrakt) nachgewiesen werden.

[1] We wish to thank Dr. C. BERNARD LEWIS of the Institute of Jamaica for supplying the specimens of *Bufo marinus*.

[2] L. C. CRAIG, J. Biol. Chem. *155*, 519 (1944).

[3] 5-Hydroxytryptamine, as the creatinine sulfate complex, was generously supplied by the Abbott Laboratories, North Chicago, Illinois, U.S.A.

[1] The radioactive tryptamine derivatives were located on the chromatograms by radioautography.

[2] The radioactivity of the samples, which were essentially weightless, was measured with a 1·8 mg/cm² mica window G. M. counter. The amounts of $I^{131}$ and $S^{35}$ in each sample were determined by measurement of radioactivity with and without an aluminum absorber as described by A. S. KESTON, S. UDENFRIEND and M. LEVY, J. Amer. Chem. Soc. *72*, 748 (1950) and S. UDENFRIEND, J. Biol. Chem. *187*, 65 (1950).

## Further Experiments on the Fixation *in vitro* of Radiocalcium to Sections of Bone

Historadiography of ground sections of total bone shows that minerals are unevenly distributed in the structures of second order of bone compacta and spongiosa, viz. the Ca content is lower in bone tissue of recent formation[1]. No changes of the degree of the X-ray absorption in recently laid down and old structures occur when organic components of bone are removed by microincineration (at 700°C for 3 h)[2] or by treatment with glycol/K hydroxide (GABRIEL's method).

The investigation has been further extended and the new data can be summarized as follows:

(1) Ground sections of total bone (40 to 50 $\mu$ in thickness) decalcified from 18 to 24 h in highly diluted

[1] A. ENGSTRÖM and R. AMPRINO, Exper. *6*, 276 (1950); R. AMPRINO and A. ENGSTRÖM, Acta Anat. *15*, 1 (1952).

[2] R. AMPRINO, Z. Zellforsch. *37*, 144 (1952).