which forms LTPP from TPP and bound LA. The loss of this enzyme is apparently the critical change resulting from mutation of the parent strain. This conclusion is based upon studies of pyruvate oxidation systems, resolved and partially purified by the method of Korkes, *et al.*,[2] from the wild and mutant strains of this organism.

Pyruvate dismutation requires transacetylase, lactic dehydrogenase, orthophosphate, diphosphopyridine nucleotide, coenzyme A, TPP, and two enzyme fractions from the wild strain (designated $A_W$ and $B_W$)[2] (Table I). However, the enzyme fraction $B_W$ and the cofactor TPP can be replaced by a single substance, LTPP, indicating that the basic pyruvate oxidase system, which is activated by the coenzyme LTPP, is present only in fraction A. The apoöxidase fraction from the mutant strain, $A_M$, can likewise be activated either by LTPP, or by TPP plus $B_W$, but *neither* apoöxidase fraction is activated by TPP plus $B_M$, the fraction from the mutant corresponding to fraction $B_W$ of the wild strain.

TABLE I

PYRUVATE DISMUTATION WITH PURIFIED ENZYME FRACTIONS

| Components[a] | μM Products in 60 min. Carbon dioxide | Acetyl phosphate | Lactate |
|---|---|---|---|
| $A_W$ + TPP | 0 | 0 | 0.2 |
| $A_W$ + $B_W$ + TPP | 3.6 | 3.4 | 3.7 |
| $A_W$ + TPP + LA | 0 | 0 | 0.2 |
| $A_W$ + LTPP | 4.7 | 4.4 | 4.7 |
| $A_W$ + Incubated[b] ($B_W$ + TPP) | 3.5 | 3.3 | 3.4 |
| $A_W$ + Control[c] | 0.1 | 0.2 | 0.2 |
| $A_M$ + TPP | 0.1 | 0.1 | 0.1 |
| $A_M$ + $B_W$ + TPP | 3.6 | 3.3 | 3.5 |
| $A_M$ + LTPP | 4.7 | 4.4 | 4.8 |
| $A_M$ + Incubated[b] ($B_W$ + TPP) | 3.5 | 3.2 | 3.4 |
| $A_M$ + Control[c] | 0 | 0 | 0.1 |
| $A_W$ + $B_M$ + TPP | 0.1 | 0.1 | 0.3 |
| $A_W$ + $B_M$ + TPP + LA | 0.1 | 0.1 | 0.3 |
| $A_M$ + $B_M$ + TPP | 0.1 | 0.1 | 0.1 |

[a] Present at following levels: enzyme fractions, 2.0 mg. protein; TPP, 100γ; LA, 10γ; LTPP, 24γ of crude synthetic preparation[1b]; final volume, 2 ml. Supplements and experimental conditions as previously described.[1a] [b] Incubated 90 min. at 25°, boiled 10 min., and supernatant added to $A_W$ or $A_M$. [c] $B_W$ incubated and boiled prior to contact with TPP.

Incubation of fraction $B_W$ alone with TPP produces a heat stable product, presumably LTPP, which can subsequently activate the apoöxidase of either strain; however, heating fraction $B_W$ prior to its contact with TPP results in an incubation mixture having no coöxidase activity. Fraction $B_W$ must furnish lipoic acid conjugase as well as lipoic acid, presumably bound to the conjugase, or less likely to a contaminating protein, by a union not dissociable by dialysis.

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(2) S. Korkes, *et al.*, *J. Biol. Chem.*, **193**, 721 (1951).

## VERATROBASINE AND GERALBINE, TWO NEW ALKALOIDS ISOLATED FROM VERATRUM ALBUM[1]

*Sir:*

After separation of the ester alkaloids, protoveratrine and veralbidine,[2] and the alkamines jervine and rubijervine from the mixture of alkaloids contained in *Veratrum album*, the mother liquor was divided into two fractions, one of which contained the markedly basic alkaloids, and the other the weakly basic alkaloids. From the fraction containing the first group an unknown alkaloid could be crystallized out of a solution in ethyl acetate.[3] This was purified as its hydrochloride which is only slightly soluble in water. After cleavage of the salt with dilute ammonia the pure base was obtained. This we intend to call *veratrobasine*. The new base crystallizes from methanol in large prisms, which turn yellow from 270° upward, and melt at 285–288°, with decomposition. Its optical rotation in pure alcohol is $[\alpha]^{20}D$ −76.6°, and in pyridine $[\alpha]^{20}D$ −126°. When veratrobasine was dissolved in 84% sulfuric acid (2 mg. of the base in 10 cc. acid) an intensely orange fluorescent solution was obtained. The solution kept this color for over 24 hours. *Anal.* Calcd. for $C_{24}H_{37}O_3N$: C, 74.44; H, 9.63; N, 3.62. Found: C, 74.46, 74.38; H, 9.81, 9.57; N, 3.66, 3.85.

In possessing only 24 carbon atoms, the new alkaloid is significantly different from the other alkamines so far obtained from *Veratrum album* and *Veratrum viride*, which all have 27 carbon atoms. Veratrobasine has one $N\text{-}CH_3$ group, the first to be found in the veratrum alkaloids: $N\text{-}CH_3$ calcd. 3.85, found 3.94.

The base also contains two active hydrogen atoms: calcd. 0.508, found 0.51.

The infrared absorption spectrum shows no band typical of ketones; the ultraviolet absorption spectrum, however, shows a definite maximum at 252 mμ (log ε 2.14).

From the fraction containing the weakly basic alkaloids, a further new alkaloid could be crystallized from ethyl acetate. This new alkaloid, which we have called *geralbine*, crystallizes from aqueous acetone in large prisms, and from a mixture of ethyl acetate and ether (1:1) in rectangular plates which melt at 221–223° with slight yellowish discoloration. In contrast to the other alkaloids isolated from *Veratrum*, geralbine exhibited no measurable rotation in pure alcohol, chloroform or pyridine. When dissolved in 84% or pure sulfuric acid a light yellow solution was obtained which had not lost its color after twelve hours. In crystalline form geralbine is fairly stable but when dissolved in alcohol or chloroform, the solution turns yellow within a few hours. *Anal.* Calcd. for $C_{22}H_{33}O_2N$: C, 76.91; H, 9.68; N, 4.07. Found: C, 76.73, 76.78; H, 9.79, 9.78; N, 3.96, 3.98.

Geralbine hydrochloride crystallizes from methanol–ether in fine needles, and melts at 270°. *Anal.* Calcd. for $C_{22}H_{34}O_2NCl$: C, 69.55; H, 9.02; Cl, 9.35. Found: C, 69.96; H, 9.23; Cl, 9.35, 9.37.

(1) Second communication. A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **35**, 1270 (1952).

(2) A. Stoll and E. Seebeck, *Science*, **115**, 678 (1952).

(3) A detailed description of the process used to isolate the two new alkaloids will be published later in *Helv. Chim. Acta*.

Like veratrobasine, geralbine has one N-$CH_3$ group: calcd. 4.38%, found 3.96%.

In the infrared spectrum geralbine shows a band typical of ketones at 1715 cm.$^{-1}$.

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## STRUCTURE OF HYALURONIC ACID. THE GLUCURONIDIC LINKAGE

*Sir:*

The isolation of a crystalline disaccharide[1] from the biologically important polysaccharide hyaluronic acid[2] has recently been reported. The glucuronic acid–glucosamine disaccharide, designated hyalobiuronic acid, has now been shown to be D-glucopyruronosido<$\beta$-1,3>D-glucosamine. This structure follows from transformation of the disaccharide to D-glucopyranosido<$\beta$-1,2>D-arabinose, a new compound whose structure follows in turn from its preparation from laminaribiose (glucopyranosido<$\beta$-1,3>glucose[3]) by an application of the Zemplén[4] degradation.

The crystalline glucuronido-glucosamine is produced from umbilical cord hyaluronic acid in yields as high as 61% by the combined enzymatic and acid hydrolysis earlier described,[1] in somewhat lower yield by direct acid hydrolysis. The picture[2] of the polysaccharide as a chain of alternating glucosamine and glucuronic acid residues must therefore be essentially correct. Also, the $\beta$-1,3-linkage now found in the disaccharide is apparently the predominating if not sole glucuronidic linkage in the polysaccharide.

In earlier structural investigations, a methylated glucopyruronoside derivative has been isolated in trace quantity on methanolysis of the methylated polysaccharide,[5] and various workers have inferred from the periodic acid consumption of the polysaccharide and its derivatives the presence of 1,3-,[6a] 1,4-[6b] or mixed 1,3- and 1,4-[6c] glucuronidic linkages.

With cold weak methanolic hydrogen chloride the glucuronidoglucosamine (I) gives an amorphous methyl ester hydrochloride (II). Acetylation of this gives heptaacetylglucuronido-glucosamine, methyl ester (III, 65% yield from I), obtained as needles, m.p. 120°, $[\alpha]^{22}D$ + 25° (chloroform), containing one ethanol of crystallization incompletely lost on drying at 110°. Found (crystals): $CH_3O$, 8.97; N, 2.08; loss on drying, 5.1. Found (dried substance): $CH_3O$, 6.05; N, 2.10; C, 48.49; H, 5.77; $CH_3CO$, 45.9; mol. wt., 668. With ketene the glucuronido-glucosamine (I) gives the amorphous N-acetyl derivative, $[\alpha]^{28}D$ −32° (water). Found: N, 3.28; uronic acid ($CO_2$), 48.2; hexosamine, 44.4. Treatment with cold weak methanolic hydrogen chloride, followed by acetylation, gives the heptaacetyl methyl ester (III) described above.

The methyl ester hydrochloride (II), on oxidation with yellow mercuric oxide, followed by sodium borohydride reduction, gives glucosido-glucosaminic acid (20% yield from I), needles, $[\alpha]^{30}D$ −34° (water; *c*, 0.9). Found: neut. equiv. (formol), 355. Degradation of this amino acid with ninhydrin gives a glucosido-arabinose, isolated as the heptaacetate (IV), needles m.p. 198–199° (micro-block), $[\alpha]^{28}D$ −47° (chloroform). Found: C, 49.92; H, 6.00. This acetate gives a melting point depression with Zemplén's[4] heptaacetyl-glucosido<$\beta$-1,3>arabinose, $[\alpha]D$ −17°, and gives no depression with heptaacetylglucosido<$\beta$-1,2>-arabinose (IV) from laminaribiose.

Synthetic laminaribiose[3,7] is treated with hydroxylamine. The resulting glass with acetic anhydride and sodium acetate at 110° gives octa-acetyllaminaribionitrile, m.p. 140–141°, $[\alpha]^{30}D$ +3° (chloroform). Found: N, 2.10. Reaction of the nitrile with sodium methoxide and acetylation of the product gives heptaacetyl-glucopyranosido<$\beta$-1,2>D-arabinose (IV), m.p. 199.5–200° (micro-block), $[\alpha]^{30}D$ −46° (chloroform). Found: C, 49.20; H, 5.62; $CH_3CO$, 47.8.

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(7) We are indebted to Prof. E. L. Hirst for seed material.