

by the original muscle pulp⁴. I have since been successful in obtaining from muscle tissue, by means of a modified purification scheme to be described in detail elsewhere, enzyme preparations catalysing the transamination of aspartic acid at a considerable rate in the presence of a thermostable activator, or coenzyme, contained in tissue extracts.

This enzyme preparation still contains the enzyme of glutamic acid trans-amination, but from vegetable materials preparations can be obtained that are active only with regard to aspartic acid while completely indifferent to glutamic acid. The aspartic enzyme differs from the glutamic enzyme in that it is more readily extracted from tissues by aqueous solvents, and also in its higher lability. The glutamic enzyme appears to contain an active group similar to the coenzyme of aspartic enzyme, but less readily dissociable. The chemical nature of the active thermostable constituent is under investigation in this laboratory.

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¹ Kritzmann, *Biochimia* (Moscow), **3**, 693 (1938).

² *C.R. Acad. Sci. U.R.S.S.*, **21**, No. 1-2, 72 (1938).

³ Braunstein and Kritzmann, *Enzymologia*, **2**, 129 (1937).

⁴ Karyagina, *Biochimia*, **4**, No. 2 (1939) (in the Press).

Purification of Uricase

URICASE is an enzyme which catalyses the oxidation of uric acid. It was reported recently by Davidson¹ that he had been able to obtain a preparation of uricase about a thousand times more active than the acetone powder of liver which was used as a starting material. Several purified preparations showed, according to him, a high and fairly constant iron content ranging from 0.15 to 0.20 per cent, which he regards as evidence in favour of the view that iron is a constituent of uricase.

Using a modified method of purification, I have been able to obtain preparations 1,000-1,400 times more active than the acetone powder of pig's liver used as initial material. My preparations are completely colourless, insoluble in water and only sparingly soluble in alkaline buffer solutions. The iron content of these preparations as determined on samples up to 16 mgm. of dry weight, is only 0.02 per cent.

Their catalytic activity is poisoned reversibly by potassium cyanide but not by hydrogen sulphide, α -dipyridyl, pyrophosphate or diethyldithiocarbamate.

The lack of proportionality between iron content and activity in preparations obtained with different methods and the very low iron content (0.02 per cent) in highly purified enzyme preparations make it very improbable that iron is a constituent of uricase.

An account of the purification and properties of the purified enzyme will be given elsewhere.

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¹ Davidson, J. N., *NATURE*, **141**, 790 (1938). *Biochem. J.*, **32**, 1386 (1938).

Marrubiin, a Diterpenoid Lactone

ACCORDING to Gordin¹, marrubiin, the optically active bitter principle of *Marrubium vulgare*, is a lactone, $C_{21}H_{28}O_4$, m.p. 154.5°-155.5°, which on hydrolysis yields marrubic acid, $C_{21}H_{30}O_5$, m.p. 173°-174°, forming an ethyl ester, m.p. 87°.

In the course of an investigation on the constitution of this compound, which has m.p. 158°, we have found that the analytical results and molecular weight determinations given by marrubiin and a number of its derivatives are in agreement with the formula $C_{20}H_{28}O_4$ for the lactone which on hydrolysis gives rise to a monobasic acid $C_{20}H_{30}O_5$, m.p. 197° (methyl ester, m.p. 85°; ethyl ester, m.p. 88°). Hydrogenation of marrubiin and marrubic acid gave the corresponding tetrahydro-derivatives, m.p. 132° and m.p. 187° (ethyl ester, m.p. 95°) respectively, which like the parent compounds are interconvertible.

Determination of active hydrogen indicates that marrubiin contains one hydroxyl group and, since the compound does not appear to form acyl derivatives under the usual conditions, this is in all probability a tertiary alcoholic group; the fourth oxygen atom is present in an oxide system. Marrubiin, which is readily resinified by warm mineral acids and by hot formic acid, gives rise on oxidation with potassium permanganate to a neutral compound, m.p. 211°, and a lactone, m.p. 161° (acid, m.p. 208°), which is also formed along with a liquid acid by the action of ozone, whilst dehydrogenation with selenium furnishes a comparatively good yield of 1:2:5-trimethylnaphthalene (agathaline). It is concluded that marrubiin is a hydroxyditerpene lactone of the manoyl oxide type.

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¹ Gordin, *J. Amer. Chem. Soc.*, **30**, 265 (1908).

Effect on Bacteria of Continued Cultivation in Lecithin Broth

THE pathogenic properties of bacteria (typhi, dysenteriae, coli) are slowly reduced by daily subcultures (about a hundred) on bouillon containing between 0.2 per cent and 4 per cent colloidal lecithin¹. For the sake of brevity these will be designated as *L*-bacteria and normal organisms as *N*-bacteria.

Particularly interesting results were obtained with *B. typhi murium*, which after four hundred subcultures required 10,000 times the original dosage to produce a fatal effect when injected intraperitoneally into mice (1,000 and 10,000,000 living bacteria, respectively). Neither fifty subsequent subcultures on ordinary media nor twelve animal passages induced a return of the pathogenic properties. The agglutinability of the *L*-bacteria with immune serum produced by the corresponding *N*-bacteria was not measurably lessened, nor was their ability to produce H and O agglutinins in affected animals.

The duration and intensity of the immunity resulting from intraperitoneal administration of *L*-strains were equal to the results observed in surviving animals infected with *N*-strains, and the results were better than those obtained by the injection of 10-100 times as many *N*-bacteria killed at 60° C.