5. On reduction in the presence of platinum, nitropyruvic ureide is converted into the oxime of hydantoin-5-aldehyde.

NEW HAVEN, CONNECTICUT

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF BRISTOL]

GALLOTANNIN. XIV. THE ACTION OF YEAST ON GALLOTANNIN

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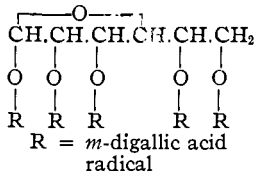
Biddle and Kelley[1] have noticed that yeast (*Saccharomyces cerivisiae*) totally destroys the optical activity of gallotannin, without apparent formation of gallic acid. We have confirmed these observations, although Geake and Nierenstein[2] failed to do so, and we have prepared by this method a gallotannin which is optically inactive and gives no glucose on hydrolysis according to Fischer and Freudenberg[3] and Feist and Haun.[4] We also find (1) that no *m*-digallic acid is produced by the action of yeast on gallotannin and (2) that only little gallic acid is formed during this process.

These results are not in agreement with the penta-digalloylglucose formula (I) of Fischer and Freudenberg.[5] Their formula requires that the *complete* conversion of gallotannin into either *m*-digallic acid or gallic acid should *precede* the production of glucose, which is, however, obviously not the case. On the other hand the polydigalloyl-leucodigallic acid anhydride formula (II) of Nierenstein[6] which is also supported by the analytical observations of Mitchell[7] and which does not exclude the existence of a gallotannin-*glucoside*[8] seems to meet the case, if one assumes provisionally that the glucose in the gallotannin-glucoside is attached to the hydroxyl, marked α in Formula II, of the asymmetric carbon atom in the leuco-digallic acid radical. Such an assumption, which is also in agreement with the fact that methylo-gallotannin yields tetramethyl glucose on hydrolysis,[9] would account for the production of an optically inactive gallotannin through yeast, probably due to racemization of the leuco-digallic acid radical and fermentation of the glucose.

[1] Biddle and Kelley, THIS JOURNAL, **34**, 919 (1912).
[2] Geake and Nierenstein, *Ber.*, **47**, 895 (1914).
[3] Fischer and Freudenberg, *Ber.*, **45**, 923 (1912).
[4] Feist and Haun, *Arch. Pharm.*, **251**, 500 (1913).
[5] Fischer and Freudenberg, *Ber.*, **45**, 917 (1912).
[6] Nierenstein, *Ann.*, **388**, 226 (1912).
[7] Mitchell, *Analyst*, **48**, 7 (1923).
[8] Nierenstein, *Ber.*, **43**, 1268 (1910).
[9] Nierenstein, Spiers and Geake, *J. Chem. Soc.*, **119**, 278 (1921).



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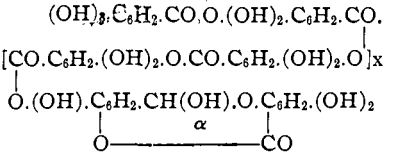

II

Experimental Part

Preparation of Yeast.—A sterilized solution of 20 g. of gallotannin in 500 cc. of water, to which is added 2 g. of a salt mixture, consisting of 5 g. of magnesium sulfate, 10 g. of ammonium phosphate, 5 g. of potassium chloride and 10 g. of sodium chloride, is inoculated with a single colony of *Saccharomyces cerivisiae*, cultivated in the usual way by a series of sub-inoculations in malt agar. The solution is kept at 37° and a vigorous fermentation, carbon dioxide being evolved, is observed after about 24 hours. The fermentation is completed in 11 days. A single colony of this yeast, cultivated in malt agar, is again inoculated into a fresh solution of gallotannin prepared as described above, and this process is repeated ten times, when a yeast of maximum intensity is obtained. This yeast, referred to later on as yeast X, produces a gallotannin with a glucose content of ±0.1% when reacting on a gallotannin containing 8.0% of glucose. During the whole process the fermentation is followed by hydrolyzing the gallotannin before and after fermentation with dil. sulfuric acid and estimating the glucose content either polarimetrically or by the reduction method of Bertrand.[10] The advance in the reactivity of the yeast increases rapidly in the first few generations, but slows down in later stages and reaches its maximum in the tenth generation.

Preparation of Gallotannin.—Chinese gallotannin, purified according to the method of Paniker and Stiasny,[11] Fischer and Freudenberg[6] and Nierenstein,[12] was used for these experiments. The gallotannins obtained by the action of yeast X on these three preparations seemed not to differ in any respect, and we are inclined to regard them as being identical.

Eight solutions of 20 g. of gallotannin prepared in the manner already described are inoculated with a single colony of yeast X. The solutions are filtered after the fermentation is completed and extracted several times with ethyl acetate. The ethyl acetate extract is shaken with a 1% solution of potassium bicarbonate, saturated with carbon dioxide and the layers are examined separately.

Aqueous Solution.—The solution is acidified with dil. sulfuric acid and exhaustively extracted with ether. The solid left on evaporation of the ether crystallizes from water, with the aid of animal charcoal, in small colorless needles, which consist of pure gallic acid, melting at 234–237°, with decomposition and evolution of gas. The product gives all the color reactions for gallic acid and the results of a combustion analysis correspond to the composition of this substance. (Found: C, 49.2; H, 3.7.) The total amount of pure, anhydrous gallic acid obtained from 129.6 g. of anhydrous gallotannin (of unknown free gallic acid content) is 22.1 g. Since these experiments were completed Mitchell[13] has described a method for the estimation of free gallic acid in gallotannin. This method, as modified in this Laboratory by Nicholson and Rhind[14] showed that a gallotannin with a free gallic acid content of 10.3% contained 11.2% of

[10] Bertrand, *Bull. soc. chim.*, [3] **35**, 1 (1906).

[11] Paniker and Stiasny, *J. Chem. Soc.*, **99**, 1819 (1911).

[12] Nierenstein, *Ann.*, **388**, 244 (1912).

[13] Mitchell, *Analyst*, **48**, 2 (1923).

[14] Nicholson and Rhind, *Analyst*, **49**, 507 (1924).

free gallic acid after the fermentation with yeast X was completed and the gallotannin had become optically inactive.

To test whether *m*-digallic acid is produced during the fermentation, 10 g. of gallotannin was fermented with yeast X and the ethereal extract methylated with diazomethane. The ethereal solution was investigated for methyl pentamethyl-*m*-digallate as described by Herzig[15] but no trace of this substance was detected.

Ethyl Acetate Solution.—The ethyl acetate solution is evaporated under diminished pressure and the solid left dissolved in a little water, which is removed in a vacuum at room temperature, and this latter process is repeated several times. The solid thus obtained resembles gallotannin in every respect in that it is precipitated by gelatin and quinine and is absorbed by caseinogen and hide powder. On analysis it gives 53.2% of carbon and 3.4% of hydrogen which agree with the values generally obtained for gallotannin. It is optically inactive in water, alcohol, acetone and ethyl acetate and gives no glucose on hydrolysis. The total amount of this gallotannin, when anhydrous, obtained from 129.6 g. of anhydrous gallotannin is 87.2 g., which corresponds to a yield of 65.7%.

In conclusion we wish to thank the Colston Society of the University of Bristol for a grant which has covered the expenses of this investigation.

Summary

A gallotannin is described which is prepared by the action of yeast on purified chinese gallotannin and is optically inactive and free from glucose.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF BRISTOL]

THE ACTION OF DIAZOMETHANE ON SOME AROMATIC ACYL CHLORIDES. III. THE MECHANISM OF THE REACTION

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In a series of communications from this[1] and the Zürich[2] Laboratories several ω-chloro-acetophenones have been described, which were prepared by the action of diazomethane on the corresponding acyl chlorides. The yields obtained by Nierenstein and his collaborators varied between 72 and 98% and it seemed therefore remarkable that only 28% of ω-bromoacetophenone is produced by the action of diazomethane on benzoyl bromide.[3]

On repeating this experiment we find that the low yield of ω-bromoacetophenone is due to the formation of 62% (average of 2 preparations) of 3,6-dibromo-3,6-diphenyl-1,4-dioxane[4] (I).

[15] Herzig, *Ber.*, **56**, 227 (1923).

[1] (a) Clibbens and Nierenstein, *J. Chem. Soc.*, **107**, 1491 (1915). (b) Nierenstein, *ibid.*, **117**, 1153 (1920). (c) Nierenstein, Wang and Warr, THIS JOURNAL, **46**, 2554 (1924). (d) Kahil and Nierenstein, *ibid.*, **46**, 2557 (1924).

[2] Staudinger and Mächling, *Ber.*, **49**, 1973 (1916).

[3] Clibbens and Nierenstein, *J. Chem. Soc.*, **107**, 1492 (1915).

[4] The name and the numbering of this ring are taken from Meyer and Jacobson, "Lehrbuch der Organischen Chemie," Veit and Co., **1920**, vol. I, pt. 3, p. 1149.