

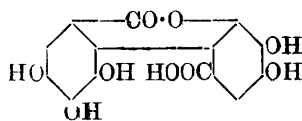
CVI.—*The Tannin of the Knopper Gall.*

By MAXIMILIAN NIERENSTEIN.

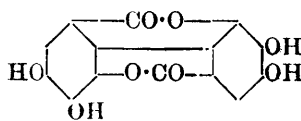
THE "Knopper Galls" (compare E. T. Connold, "Plant Galls of Great Britain," 1909, 254) or "Knoppfern," as they are known in the tanning industry (compare H. R. Procter, "The Principles of Leather Manufacture," 1903, 262), are the galls produced by *Cynips calicis*, Burgsdorff, on the immature acorns of various species of oaks, principally *Quercus cerris* of the Slavonic plains, where these galls are known locally by their Slavonic and Hungarian names, "Šišarca" and "gubacs" respectively.\* Their occurrence in Great Britain on *Quercus pedunculata* and also *Quercus sessiliflora* has been recorded by E. T. Connold ("British Oak Galls," 1908, 143), who refers to them also as "Gall-nuts." Löwe (*Zeitsch. anal. Chem.*, 1875, **13**, 46), who has carried out the only published investigation on knoppfern tannin, described it as being identical with gallotannin. This, however, is not the case, as will be seen from the present communication. It is found that knoppfern tannin yields on hydrolysis, in addition to dextrose, not gallic acid, as is the case with gallotannin, but mainly ellagic acid (II) in common with other "bloom" (ellagic acid) producing

\* I am indebted for some of this information to Dr. Jan Jedlička, whom I have also to thank for the knoppfern used in this investigation. Dr. Jedlička drew my attention to the ease with which the knoppfern extracts deposited ellagic acid which he thought was due to the presence of a ferment in these galls, since he had succeeded in preventing the formation of ellagic acid by prolonged boiling of the extracts. I have attempted to isolate this ferment, but without success. In this connexion I may mention that I also found that emulsin has no effect on knoppfern tannin, which is of interest since this ferment readily hydrolyses the tannin of *Terminalia chebula* into dextrose, luteolic acid, and ellagic acid (*Ber.*, 1910, **43**, 1268). On the other hand, I found that *Penicillium* produces ellagic acid from knoppfern tannin which corresponds with the formation of gallic acid from gallotannin by *Penicillium* (compare Knudsen, *J. Biol. Chem.*, 1912, **14**, 159; Asai, *J. Coll. Sci. Tokyo*, 1918, **39**, 23).

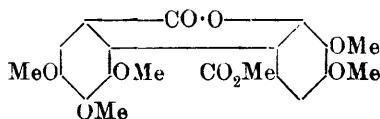
tannins (compare A. G. Perkin and Nierenstein, T., 1905, **87**, 1412). It is also observed that methylated knoppern tannin yields on hydrolysis a mixture of partly methylated luteoic acids (I), which give on further methylation with diazomethane methyl pentamethoxyluteoate (III). The latter substance was found to be



(I.)



(II.)



(III.)

identical with the lactone of methyl 2-hydroxy-3:4:4':5':6'-penta-methoxydiphenyl-6:2'-dicarboxylate (III), previously described by Herzig and Polack (*Monatsh.*, 1908, **29**, 263). From these observations it is provisionally concluded that the knoppern tannin molecule is composed of luteoic acid (I) and dextrose, the luteoic acid being predominant. Luteoic acid, which is an oxidation product of gallotannin (*Ber.*, 1908, **41**, 3015), has also been found to be the predominant component of the tannin of *Terminalia chebula* (*Ber.*, 1910, **43**, 1267). Reference ought also to be made to the presence of galloyl-*l*-leucine in the fat extracted from the knopper gall (*Zeitsch. physiol. Chem.*, 1914, **92**, 53), and the production of ellagic acid from galloylglycine by *Penicillium* (*Biochem. J.*, 1915, **9**, 240), which observations, in conjunction with the suggested configuration of knoppern tannin, may be regarded as further evidence in support of the assumption made (*Biochem. J.*, 1915, **9**, 241) that some nitrogenous galloyl derivatives serve as foodstuffs for the growing, gall-producing parasites and at the same time lead to the formation of "pathological" tannins, such as gallo-tannin, knoppern tannin, and the other tannins found in galls (compare Nierenstein, *Ber.*, 1910, **43**, 628; Drabble and Nierenstein, *Biochem. J.*, 1907, **2**, 96; Nierenstein and Webster, *Ber.*, 1908, **41**, 80).

#### EXPERIMENTAL.

##### *Preparation of Knoppern Tannin.*

The powdered and finely sieved knoppern were at first extracted with boiling chloroform or *s*-tetrachloroethane, so as to remove the

## 1176 NIERENSTEIN: THE TANNIN OF THE KNOPPER GALL.

so-called gall-fats.\* The carefully dried powder was subsequently extracted with boiling acetone. In both cases the extraction was carried out in the extraction apparatus described elsewhere (Abderhalden, "Handbuch der biochemischen Arbeitsmethoden," 1912, **6**, 149). The acetone extract was made up to 1.5 litres and the tannin precipitated with 300 c.c. of light petroleum which had been dried over calcium chloride. The crude tannin obtained in this way was purified by dissolving 50 grams of it in 1 litre of acetone and precipitating with four successive quantities of light petroleum of 100 c.c. each. It is necessary to dry both the acetone and the light petroleum over calcium chloride for some time, as otherwise the products obtained are viscid (compare also Manning and Nierenstein, this vol., p. 666). The fractions obtained weighed 26, 12, 6, and 2.5 grams respectively and were pale-coloured, amorphous products which neither melted nor decomposed when heated above 300°. They were soluble in alcohol, acetone, ethyl acetate, acetic acid, or water, but insoluble in benzene, light petroleum, or chloroform. The different solutions gave greenish-blue colorations with ferric chloride, which were particularly well-marked when the alcoholic solution was employed. This is of special interest in view of the generally accepted classification of the tannins into catechol and pyrogallol groups, which classification is based on the green and blue colorations given by these two groups respectively with ferric chloride. In this connexion it ought to be mentioned that the tannins of *Terminalia chebula* (Nierenstein, *loc. cit.*) and *Polygonum bistorta* (Stenhouse, *Phil. Mag.*, 1843, [iii], **23**, 335; Eissfeld, *Annalen*, 1854, **92**, 109; 1859, **111**, 217; Bjälöbreshesky, *Pharm. J.*, 1900, **22**, 3; Jljín, *Diss.*, Petrograd, 1905, p. 38) show similar mixed colorations with ferric chloride. The aqueous solution of knoppern tannin precipitates alkaloids and gelatin, and is quantitatively adsorbed by caseinogen (compare Manning and Nierenstein, this vol., p. 667). Knoppern tannin is hygroscopic, and the determination of its elementary composition by combustion requires the same precautions as used by Geake and Nierenstein (*Ber.*, 1914, **47**, 897) in the analysis of gallotannin.

## Found:

Fraction I.—C=54.3; H=2.1. M.W. (in acetone)=1628, 1654, 1708. M.W. (in alcohol)=1744, 1682, 1664.

\* Preliminary investigations of the gall-fats of the knoppern seem to indicate that they are identical with the fats derived from Aleppo galls (compare Kunz-Krause and his collaborators, *Apoth. Zeit.*, 1897, **37**, 734; *Arch. Pharm.*, 1904, **237**, 256; 1907, **245**, 28). These observations refer mainly to cyclogallipharic acid of the knoppern gall-fat, which had been obtained by Kunz-Krause's method. It crystallised, like his cyclogallipharic acid, from light petroleum in large scales melting at 88—89°.

Fraction II.— $C=54.1$ ;  $H=2.2$ .

Fraction III.— $C=55.4$ ;  $H=2.4$ . M.W. (in acetone)=1758, 1744, 1694. M.W. (in alcohol)=1742, 1716, 1758, 1708.

Fraction IV.— $C=55.6$ ;  $H=2.5$ . M.W. (in acetone)=1702, 1744, 1726. M.W. (in alcohol)=1758, 1744, 1726.

For the polariscopical investigations of knoppern tannin only 0.5 per cent. solutions were used in view of the experiences of Navassarat (*Kolloid Zeitsch.*, 1913, **12**, 97) in the case of gallo-tannin:

Fraction I.— $[\alpha]_D^{17} + 31.8^\circ$  (in alcohol);  $+8.4^\circ$  (in acetone).

Fraction IV.— $[\alpha]_D^{17} + 36.2^\circ$  (in alcohol);  $+11.8^\circ$  (in acetone);  $+28.6^\circ$  (in water).

### *Hydrolysis of Knoppern Tannin.*

About 0.5 gram of knoppern tannin (Fractions III and IV only were used) was dissolved in 200 c.c. of water and heated with 20 c.c. of a 5 per cent. solution of sulphuric acid for eight to twelve hours on a water-bath, a reflux condenser being used. The ellagic acid formed was collected in a Gooch crucible and washed at first with cold water, so as to remove all traces of sulphuric acid, and afterwards with alcohol and ether. The precipitate was dried at  $130^\circ$  and weighed as ellagic acid. (Found,  $C=56.4$ ,  $55.8$ ;  $H=2.1$ ,  $2.2$ . Calc.,  $C=55.6$ ;  $H=2.0$  per cent.) The filtrate was tested for dextrose by Fischer and Freudenberg's method (*Ber.*, 1912, **45**, 915) and the dextrose was estimated volumetrically by Bertrand's method (*Bull. Soc. chim.*, 1906, [iii], **35**, 1286) as used by Nierenstein and Geake (*loc. cit.*) for the estimation of dextrose in gallo-tannin. In addition to this, the dextrose was also estimated polariscopically, in one case after the hydrolysis of 12.8 grams of knoppern tannin. The dextrosazone was also prepared and found to have the correct melting point. (Found,  $N=15.9$ . Calc.,  $N=15.6$  per cent.)

Found: Ellagic acid= $84.6$ ,  $72.4$ ,  $81.2$ ,  $78.4$ . Dextrose= $7.2$ ,  $6.4$ ,  $5.8$ ,  $6.7$  (by Bertrand's method),  $7.8$  (polariscopically) per cent.

### *Methylated Knoppern Tannin.*

The methylation (Fractions III and IV only were used) was carried out with diazomethane in ethereal suspension, and the same precautions were taken as in the case of hemlock tannin (compare Manning and Nierenstein, *loc. cit.*). The methylated knoppern tannin dissolved completely in the ether and was purified several

## 1178 NIERENSTEIN: THE TANNIN OF THE KNOPPER GALL.

times by dissolving it in alcohol and precipitating with water. The methylated knoppern tannin obtained in this way was a colourless, amorphous product without any definite melting point. It was soluble in alcohol, acetone, chloroform, or *s*-tetrachloroethane, but insoluble in water or light petroleum.

Found: C=58.8, 57.9, 58.2; H=4.1, 4.4, 4.3; OMe=36.9, 37.4, 37.2, 37.8. M.W. (in alcohol)=1822, 1848, 1912. M.W. (in acetone)=1894, 1826, 1858.

The methoxyl estimations were carried out in a Zeisel-Perkin apparatus, acetic anhydride being added. If the latter was replaced by phenol (compare Weishut, *Monatsh.*, 1913, **33**, 1165) the same result (OMe=36.5) was obtained.

For the polariscopical investigations only 0.5 per cent. solutions were used, and the results obtained were as follows:

Found:  $[\alpha]_D^{20} + 4.2^\circ$  (in alcohol);  $+ 6.8^\circ$  (in acetone);  $+ 16.2^\circ$  (in chloroform);  $+ 28.2^\circ$  (in *s*-tetrachloroethane).

*Alkali Hydrolysis of Methylated Knoppern Tannin.*

Methylated knoppern tannin (about 5–10 grams) was hydrolysed by boiling with a 10 per cent. solution of alcoholic potassium hydroxide for eight hours, a reflux condenser being used. The alcohol was then evaporated and the residue extracted with boiling water, the extract being filtered from any unchanged or tarry material. On acidifying the cold solution with dilute sulphuric acid, there was obtained a bulky, colourless precipitate (Precipitate A), which was collected, washed, and dried in a desiccator. The filtrate was extracted several times with ether, which left on evaporation a tarry residue. Several attempts were made to isolate a definite disintegration product from this residue, but without success. The average yield of this residue corresponded with about 22 per cent. of the material hydrolysed. It did not reduce Fehling's solution.

*Precipitate A.*—This product proved to be a mixture, and it was found possible to separate it into two parts, respectively soluble and insoluble in benzene. The carefully dried substance was extracted in a Soxhlet apparatus with benzene, until a few drops of the overflow left no residue on evaporation. Five grams of the precipitate left about 2 grams in the thimble (Fraction I). The benzene solution gave on evaporation a second product (Fraction II). Both fractions were investigated separately.

*Fraction I.*—This product had no definite melting point (67–84°), and it was found impossible to crystallise it. The investigation of this amorphous material disclosed the following interest-

ing points, which might, perhaps, in time, prove of importance in the chemistry of knopperr tannin. It was found that the alcoholic solution gave a pronounced green coloration with ferric chloride, thus indicating (1) that it contained two hydroxyl groups in the ortho-position, and (2) that these two hydroxyl groups escaped methylation during the action of diazomethane on knopperr tannin. Attempts were also made to oxidise the alcoholic solution with silver oxide (Willstatter and Pfannenstiel, *Ber.*, 1904, **37**, 4744) or *p*-benzoquinone (A. G. Perkin, *T.*, 1913, **103**, 661; Nierenstein, *T.*, 1915, **107**, 860) in the hope of obtaining the corresponding *o*-quinone. These experiments, however, only gave negative results.

Found: C=56.8, 57.1; H=4.4, 4.2; OMe=27.8, 26.4, 27.3.

These analytical data indicated that Fraction I was still a mixture consisting of tetramethoxyluteoic acid (Calc., C=57.4; H=4.2; OMe=32.9 per cent.) and trimethoxyluteoic acid (Calc., C=56.3; H=3.8; OMe=25.6 per cent.), the latter being predominant.

That Fraction I was a mixture was also proved on acetylation with acetyl chloride and pyridine. The acetyl derivative was also amorphous, and decomposed on heating at about 129—135°. Only acetyl estimations by A. G. Perkin's method (*T.*, 1905, **87**, 107) were made, which gave Ac=14.5, 13.8, 14.2 per cent., whereas the mono- and the di-acetyl derivatives require Ac=10.2 and 19.3 per cent. respectively.

On further methylation with diazomethane in ethereal solution Fraction I was entirely converted into methylpentamethoxyluteoate (III), which crystallised from alcohol in small needles melting at 110°; this is in the vicinity of the melting point, 109—111°, given by Herzig and Polack (*loc. cit.*) for this substance. For comparison, the acid was also prepared by the method described by these authors from ellagic acid, when it was also found to melt at 110°. There was no depression of the melting point when the two preparations were mixed. (Found, C=59.1; H=5.1; OMe=46.3.  $C_{20}H_{20}O_9$  requires C=59.4; H=4.9; OMe=46.1 per cent.)

*Fraction II.*—This fraction did not give clearly the colour reaction with ferric chloride mentioned above, but the analytical data obtained for it agreed on the whole better for tetramethoxyluteoic acid than for trimethoxyluteoic acid, although in view of the amorphous character of the product and its indefinite melting point, which was found to be 98—112°, it seems obvious that the material was not pure. (Found, C=57.8, 57.6; H=4.5, 4.7; OMe=31.7, 31.4, 31.6.  $C_{18}H_{16}O_9$  requires C=57.4; H=4.2; OMe=32.9 per cent.)

It was found possible to acetylate Fraction II with acetyl chloride and pyridine, but a satisfactory product was not obtained. (Found, Ac=11·8, 12·2, 12·0. Calc., Ac=10·2 per cent.)

On further methylation with diazomethane, Fraction II was entirely converted into methyl pentamethoxyluteoate (III); small needles melting at 110° were obtained, and this melting point was not changed by admixture with Herzig and Polack's product. (Found, C=59·2; H=4·9; OMe=46·1. Calc., C=59·4; H=4·9; OMe=46·1 per cent.)

This investigation has, with many interruptions, been in progress since 1907, during which time the author has had the collaboration of Dr. D. Spence and Messrs. R. J. Thompson, J. F. Fielding, C. W. Spiers, and F. S. Wallis, for which he wishes to thank them. The author is also indebted to the Research Fund Committee of the Chemical Society and the Colston Society of the University of Bristol for grants in aid of this investigation.

BIO-CHEMICAL LABORATORY,

CHEMICAL DEPARTMENT,

UNIVERSITY OF BRISTOL.

[Received, August 22nd, 1919.]

---