

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

THE CATECHIN OF THE CACAO BEAN¹

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More than twenty years ago A. J. Ultée and W. van Dorssen detected in fresh cacao beans a phenolic crystalline substance which they called "Kakaoöl." They isolated it as a crystalline caffeine compound and suggested the formula $C_{16}H_{16}O_6$. The substance forms a crystalline acetyl compound with the melting point 153–154°.

From the description of this labile material, one of us concluded² that it belonged to the catechin class. When later the various isomeric forms of catechin became better known, the additional suggestion could be made that this substance very probably represented *l*-epicatechin.³

This prediction has now been proved to be correct. We prepared the catechin from a sample of fresh cacao pods procured from Trinidad (collected in January), and another procured from Porto Rico (collected in January). A third sample was kindly sent to us by Dr. A. Frey-Wyssling of Medan, Sumatra, and consisted of fresh beans sterilized in Medan. For the fourth sample we are indebted to Dr. A. J. Ultée, who supplied us with some of his original "caffeine-kakaoöl." From all these samples the same substance was obtained. It was identical in every respect with *l*-epicatechin, which was isolated some years ago from the wood of *Acacia Catechu*.⁴

While our present work was in progress, a paper by W. B. Adam, F. Hardy and M. Nierenstein⁵ appeared in which the view was adopted that cacaoöl belongs to the catechin family. Their experimental results are in sufficient agreement with ours, and they identify it, as we do, with the levorotatory catechin present in the catch-producing acacias; but they call it *l*-acacatechin and ascribe to it a constitutional formula which we believe is incorrect.

We maintain that catechin and epicatechin are stereoisomeric and that both have the formula I. The tetramethyl ether of each gives rise, after reduction and methylation, to the same pentamethoxy-1,3-diphenylpropane (II).⁶ *dl*-Epicatechin has been obtained from cyanidin⁷ by hydro-

¹ Researches on Tannins, No. 26; preceding communication, *Ann.*, **483**, 140 (1930).

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² K. Freudenberg, *Ber.*, **53**, 1416 (1920).

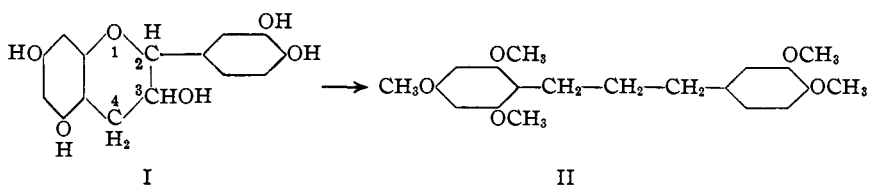
³ K. Freudenberg and L. Oehler, *Ann.*, **483**, 141 (1930).

⁴ Freudenberg, Fikentscher, Harder and Schmidt, *ibid.*, **444**, 139 (1925).

⁵ Adam, Hardy and Nierenstein, *THIS JOURNAL*, **53**, 727 (1931).

⁶ See *Ann.*, **451**, 213 (1926), Ref. 2 for references.

⁷ Ref. 4, page 135.



genation, and its pentamethyl ether from pentamethylcyanidin and pentamethylquercetin⁸ in the same way. As the formulas of cyanidin and quercetin are definitely established, no doubt seems possible concerning the constitution of epicatechin.

Synthetic epicatechin is identical with a mixture of equal parts of natural *l*-epicatechin and *d*-epicatechin obtained by the epimerization of natural *d*-catechin.⁹ *dl*-Epicatechin forms by epimerization *dl*-catechin⁹ which is identical with a mixture of equal parts of natural *d*-catechin and *l*-catechin formed by the epimerization of *l*-epicatechin.¹⁰ *dl*-Catechin in its turn can be converted into *dl*-epicatechin.¹¹ The scheme of these relationships given in 1924,¹² can be completed by an arrow leading from *dl*-epicatechin to *dl*-catechin. Catechin and epicatechin are, therefore, stereoisomers, and their constitution is expressed by the same formula (I). The fact that both yield the same 1,3-diphenylpropane derivative (II) leads to the same conclusion.

It has been deemed necessary to develop a part of the evidence given in favor of formula I for both catechin and epicatechin as M. Nierenstein¹³ has been attempting for many years to establish another formula in which the catechol nucleus is attached to carbon atom 3 or 4 (I). He bases his suggestion on his failure to obtain substance II from either catechin or epicatechin.

As a matter of fact, M. Nierenstein has omitted to carry out, in accordance with our procedure,⁶ this fundamental reaction leading from I to II, in spite of the various suggestions which have been made to him to perform this simple experiment.¹⁴ We, therefore, confine ourselves to a few criticisms of some of his later papers.

⁸ Freudenberg and Kammüller, *Ann.*, **451**, 209 (1927).

⁹ Ref. 7, page 140.

¹⁰ Freudenberg and Purrmann, *Ann.*, **437**, 283 (1924).

¹¹ Freudenberg, *Ber.*, **55**, 1738 (1922); Freudenberg and Purrmann, *ibid.*, **56**, 1190 (1923).

¹² See Ref. 10, page 275; Freudenberg and Harder, *Ann.*, **451**, 214, Ref. 3 (1927).

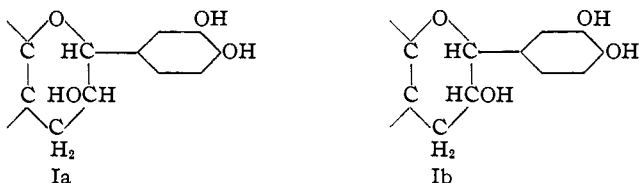
¹³ Nierenstein, *Ber.*, **56**, 1877 (1923); *THIS JOURNAL*, **46**, 2793 (1924); *ibid.*, **48**, 1959 (1926); *ibid.*, **52**, 1672 (1930); *Chem. Age* (London), **19**, 291, 361 (1928); *J. Indian Chem. Soc.*, **7**, 279 (1930); for earlier quotations *cf.* *Ber.*, **56**, 2128, Ref. 5 (1923); *Ann.*, **437**, 279, Ref. 2 (1924); and the critical remarks, *Ber.*, **56**, 1188, Ref. 6 (1923); and *ibid.*, **55**, 1938 (1922).

¹⁴ Nierenstein, *ibid.*, **56**, 1188, Ref. 6 (1923); *ibid.*, **55**, 1938 (1922); finally K. Freudenberg, some years ago, deposited authentic material in another laboratory and asked Dr. Nierenstein to write to this Laboratory for a part of this substance and carry out

M. Nierenstein and E. O. Hazelton¹⁵ tried to convert *dl*-catechin into *dl*-epicatechin and from their failure concluded that these substances cannot have the same structural formula. They overlooked the fact, mentioned above, that this conversion had been accomplished nine years before.

M. Nierenstein¹⁶ is laboring under a misapprehension in assuming that the work of J. J. Drumm, R. J. P. Carolan and H. Ryan¹⁷ proves a formula of catechin proposed by him and disproves our formula (I). The Irish authors, on the contrary, expressly state that their result is in agreement with our formula.¹⁸

Furthermore, M. Nierenstein¹⁹ refers to a remark made by W. Hüchel²⁰ as being a criticism of our formula. Some time ago²¹ one of us suggested that, of the two stereoisomeric formulas derived from formula I, epicatechin has the spacial arrangement Ia and catechin Ib. Evidence for this de-



cision seemed to be given by the fact that tetramethylepicatechin readily loses water, while tetramethylcatechin splits off water only with difficulty, undergoing a rearrangement at the same time. Later²² we again left open the question as to which formula of the two, Ia or Ib, must be ascribed to catechin or epicatechin. Two years later Hüchel, who had overlooked the last remark, made the same suggestion. The criticism of Hüchel was therefore unnecessary, and did not touch at all upon the question of constitution as might be inferred from Nierenstein's remark.

We state once more that all catechins which K. Freudenberg and his collaborators have had in hand thus far, are stereoisomeric catechins or epicatechins and are all to be expressed by formula I.

Experimental

The beans used in this investigation were heated for an hour at 75° in 95% alcohol, immediately after removal from the pods, in order to kill enzymes. After air-drying the experiment. Cf. R. Robinson's remarks on the question [*Chem. Age* (London), 19, 337 (1928)].

¹⁵ Nierenstein and Hazelton, *J. Indian Chem. Soc.*, 7, 279 (1930).

¹⁶ Nierenstein, *THIS JOURNAL*, 53, 1500 (1931).

¹⁷ Drumm, Carolan and Ryan, *Proc. Royal Irish Acad.*, [B] 39, 114 (1929).

¹⁸ Cf. R. Robinson, *Chem. Age* (London), 19 (1928).

¹⁹ Ref. 16, page 1501.

²⁰ Hüchel, *Ann.*, 477, 159 (1929).

²¹ Freudenberg, Fikentscher and Wenner, *Ann.*, 442, 309 (1925); Freudenberg, Carrara and Cohn, *ibid.*, 446, 87 (1925).

²² Freudenberg and Harder, *ibid.*, 451, 214 (1927).

they were ground in a mill and the catechin removed by maceration with alcohol at 75°. The alcohol solution contained fat, caffeine and catechin. After removal of the alcohol under reduced pressure, the residue, dissolved in a little water, was extracted a few times with petroleum ether for removal of fat, and then exhaustively extracted with chloroform to remove the caffeine. The resulting deep red solution was extracted with ether for several days. The solid remaining after evaporation of the ether was taken up in a small quantity of boiling water, treated with talcum to absorb any fat that might be present, and allowed to crystallize. After several days, small rosetts of thick prisms had crystallized from the solution. These were of a slight brownish-pink tinge and had to be purified from an acetone-benzene solution.²³

The material obtained from Trinidad and Porto Rico was sterilized after arrival in Madison, whereas the beans from Sumatra were sterilized there. The meat of the latter beans was light pink, while the meat of the former two samples was dark brown. The yield obtained from the first two samples was only one to two-tenths while the yield from the Sumatra beans was five-tenths of one per cent.

Two and three-tenths grams of the compound sent by Dr. Ultée was dissolved in 25 cc. of hot water, filtered to remove solid impurities and extracted fifteen times in a separatory funnel with 15-cc. portions of chloroform. The water layer was then heated to boiling, filtered and set aside to crystallize. It was seeded with *l*-epicatechin, and the next day a crop of crystals was obtained.

The crystalline form and solubilities of the four samples did not differ from those of *l*-epicatechin. The crystals lost four molecules of water at 100° under reduced pressure (av. 19.5%, calcd. 19.9%).²⁴ The water of crystallization is lost slowly at room temperature.

When heated, following the procedure of E. Fischer,²⁵ the substances decomposed at 237–239° (corr. 245°). *l*-Epicatechin itself and mixtures of the substances with *l*-epicatechin behaved likewise.²⁶

The rotation of the catechin of cacao was measured in acetone-water (1:1 by weight): $[\alpha]_D^{20} -0.58 \times 100/1 \times 0.998 = -58^\circ$. For *l*-epicatechin in yellow mercury light, $-59-60^\circ$ is indicated.²⁷ (M. Nierenstein⁵ used water and found -69° .)

Anal. Subs., 4.368 mg.: CO₂, 9.98; H₂O, 1.99. Calcd. for *l*-epicatechin: C, 62.07; H, 4.82. Found: C, 62.81; H, 5.09.

The acetyl compound was prepared in the usual way.²⁸ The melting point of pentacetyl-*l*-epicatechin was 151–152° (corr. 153–154°).²⁹ When mixed with the original preparation the melting point did not alter.

The acetates of our cacao-catechin as well as the acetate of Dr. Ultée's material had the same rotation in acetylene tetrachloride, varying from -12.1 to -12.3° ; $[\alpha]_D^{20} -1.09 \times 100/1 \times 8.84 = -12.3$.

Anal. Subs., 4.332 mg.: CO₂, 9.51; H₂O, 1.87. Calcd. for pentacetyl-*l*-epicatechin: C, 59.98; H, 4.83. Found: C, 59.87; H, 4.83.

²³ Ref. 4, page 144.

²⁴ Ref. 10, page 276.

²⁵ Fischer, *Ber.*, **41**, 75 (1908).

²⁶ The point of decomposition of 245° as indicated in *Ann.*, **437**, 276 (1924), is corrected (*cf.* *Ber.*, **56**, 1192 (1923)). M. Nierenstein (ref. 5) indicated a melting point of 229°. When heated slowly, this point of decomposition can also be obtained with our substances.

²⁷ Freudenberg and Purrmann, (a) *Ann.*, **437**, 276 (1924); (b) *Ber.*, **56**, 1192 (1923).

²⁸ Freudenberg, Böhme and Beckendorf, *ibid.*, **54**, 1209 (1921).

²⁹ Freudenberg and Purrmann, *ibid.*, **56**, 1189 (1923); *Ann.*, **437**, 276 (1924).

In a 2-3% solution with mercury light, specific rotations of -14.5 to -15.0° were found for *l*-epicatechin pentacetate. M. Nierenstein reports -12° for sodium light (concentration not indicated).

The melting points reported by M. Nierenstein and his collaborators³⁰ for the acetates of the so-called *dl*-acacatechin (160°), *d*-gambircatechin (137°), *dl*-gambircatechin (156°) do not correspond to those for our pure substances. The acetates of *dl*-epicatechin, *d*-catechin and *dl*-catechin melt at 169° , $131-132^\circ$, $164-165^\circ$, respectively.^{28a}

Summary

1. The catechin present in the cacao bean is shown to be identical with *l*-epicatechin.
2. Recent work of Dr. Nierenstein is criticized.

³⁰ Ref. 5, p. 1504.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE COLLEGE OF THE OZARKS]

THE PREPARATION OF 5,7-DI-iodoisatin

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The preparation of 5,7-di-iodoisatin has been described by Kalb and Berrer.¹ These investigators treated an aqueous solution of the sodium bisulfite addition compound of dehydroindigo with iodine monochloride. 5,7-Di-iodoisatin was obtained by treating the resulting solution with a solution of potassium dichromate in aqueous sulfuric acid.

The writers attempted to repeat the work of Kalb and Berrer but were able to obtain only very poor yields of the compound by this method. A procedure was then developed for preparing 5,7-di-iodoisatin from 5,7,5',7'-tetraiodoindigo. The latter compound was oxidized with an aqueous solution of chromic and nitric acids and more satisfactory yields of the di-iodoisatin were obtained.

It has also been stated that 5,7-di-iodoisatin,² as well as a small amount of tetraiodoisatin, is obtained when a solution of isatin in concentrated hydrochloric acid is treated with iodine monochloride. Hill and Sumpter found that this statement³ is in error, 5-iodoisatin being the sole product even when a large excess of iodine monochloride is used.

The writers have further attempted to prepare 5,7-di-iodoisatin by the action of iodine monochloride on isatin and on iodoisatin in methyl alcohol, ethyl alcohol, concentrated sulfuric acid, fuming sulfuric acid and acetic anhydride without success. It appears that iodine cannot be introduced

¹ Kalb and Berrer, *Ber.*, **57**, 2112 (1924).

² German Patent 429,101.

³ Sumpter, Ph.D. Dissertation, Yale University, 1930.