

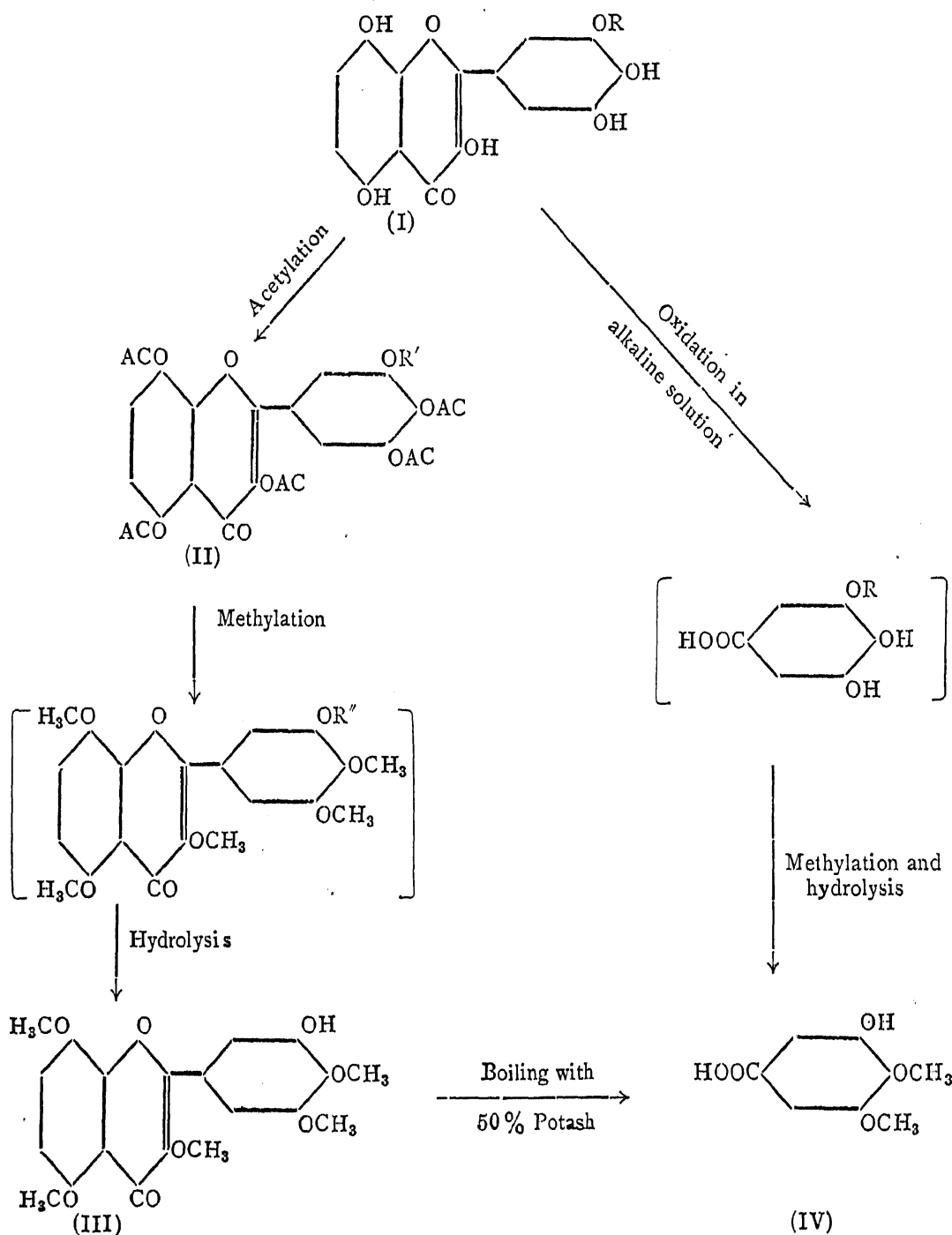
CONSTITUTION OF CANNABISCITRIN

BY P. SURYAPRAKASA RAO AND T. R. SESHADRI
(From the Department of Chemistry, Andhra University, Waltair)

Received August 5, 1941

It has recently been shown that the colouring matter of the flowers of *Hibiscus cannabinus* consists of a new flavonol called cannabiscetin and its glucoside, cannabiscitrin, the latter being the main component. From a study of its reactions and the degradation products, the constitution of the flavonol has been deduced to be 3:5:8:3':4':5'-hexahydroxyflavone.¹ Cannabiscitrin is concluded to be a monoglucoside from the combustion analysis and from the yields of the products of hydrolysis. In giving an orange-red precipitate with lead acetate and in being hydrolysed with difficulty by acids, the new glucoside ($C_{21}H_{20}O_{13}$) resembles gossypitrin, herbacitrin and quercimeritrin which carry the sugar groups in position 7, and this behaviour shows that it is not a 3-glucoside. Neither the 5- nor the 8-hydroxyl group seems to be involved in the glucoside-formation, since the substance undergoes ready oxidation to give the "gossypetone" reaction. It may, therefore, be concluded that the sugar group is present not in the benzopyrone ring but in the side phenyl nucleus. This surmise has been substantiated and the exact position involved determined in the following manner: Cannabiscitrin is completely methylated through the acetyl derivative² (II) and the product hydrolysed in order to remove the sugar part. When the resulting pentamethyl cannabiscetin is decomposed with boiling 50% potash, a dimethyl gallic acid is obtained. This is different from syringic acid, and found to be identical with 4:5-dimethyl gallic acid (IV). The same compound is also obtained by subjecting the glucoside to aerial oxidation in cold alkali, directly methylating the products and finally effecting the removal of the sugar molecule by acid hydrolysis. Cannabiscitrin is, therefore, concluded to be the 3'-glucoside (I), and the pentamethyl-ether mentioned above to be 3:5:8:4':5'-pentamethyl cannabiscetin (III). The changes involved are represented diagrammatically below.

Thus cannabiscitrin is unusual amongst glycosides of flavones and flavanols in holding the glucose residue in the side phenyl nucleus. The cases so far known belonged to only two categories (1) the 3-glycosides and (2) the 7-glycosides. Recently it has been reported by us³ that in butrin the flavanone structure carries one of the glucose units in the 3'-position, and



(R is glucose residue and R' is the fully acetylated sugar group)

hence this type of glycosides seems to be more common than originally thought of.

Experimental

Hydrolysis of Cannabiscitrin: Estimation of the Aglucone and the Glucose.—The glucoside (2 g.) was hydrolysed by boiling with 7% sulphuric acid (100 c.c.). After heating for a few minutes the solid went into solution, and after twenty minutes the aglucone began to separate out, giving rise to bumping. The heating was, therefore, continued on a boiling water-bath

for an hour and a half more. The contents were cooled and filtered. The filtrate was almost colourless and did not yield any appreciable amount of the aglucone on ether extraction. So the residue after the filtration was dried in air, and weighed accurately. The acid filtrate which contained the glucose was made slightly alkaline by the addition of the requisite amount of sodium hydroxide, made up to a known volume, and the sugar estimated by titration against standard Fehling solution.

The amounts of the aglucone and the glucose produced as a result of the hydrolysis of the glucoside were calculated. [Found: Aglucone, 63.4; Glucose, 33.6; $C_{21}H_{20}O_{13}$, 3 H_2O requires Aglucone ($C_{15}H_{10}O_8$, H_2O), 62.9; Glucose, 33.7%.]

Gossypetone Reaction with the Glucoside.—The substance (0.5 g.) was dissolved in absolute alcohol (30 c.c.) and *p*-benzoquinone (0.2 g.) in the same solvent (2 c.c.) was added. The original solutions were both yellow in colour, and the mixture immediately assumed a dark red colour, and a dull red solid began to separate out gradually. The next day it was filtered and washed with a small quantity of absolute alcohol. It was maroon-coloured, looking like red phosphorus, and did not melt below 300° . When treated with a drop of dilute potassium hydroxide, it dissolved producing a blue solution, which became red on acidification. When the substance was suspended in water and reduced with sulphur dioxide, the original glucoside was regenerated.

Methylation of the Glucoside and Subsequent Hydrolysis: Isolation of 3:5:8:4':5'-Pentamethyl Cannabiscetin.—Cannabiscitrin acetate (1 g.) was dissolved in acetone (40 c.c.) and dimethyl sulphate (10 c.c.) and 20% sodium hydroxide (10 c.c.) were added to the solution alternately in small quantities. The mixture was shaken vigorously after each addition, when it assumed a lemon yellow colour which faded gradually. Thereupon further quantities of dimethyl sulphate (5 c.c.) and the sodium hydroxide (5 c.c.) were added and finally the medium was made definitely alkaline by the gradual addition of 10 c.c. more of the above alkali. There was a gradual development of heat throughout the process, and during the later stages the mixture became so hot that there was actual boiling. After leaving overnight, it was gently boiled under reflux on a water-bath for an hour. Then almost all the solvent was driven off, the alkaline solution was just neutralised, and then sufficient amount of concentrated sulphuric acid was added so as to render the solution 7% acid. It was finally boiled under reflux for 2 hours. As no solid separated out on cooling, the contents were repeatedly extracted with ether. On evaporating off the solvent, the ether extract yielded an

almost colourless substance. When crystallised from dilute alcohol using a little animal charcoal, it was obtained as narrow rectangular plates in fern-like structures, melting at 191–92°. [Found: in the sample dried at 120° *in vacuo*: OCH₃, 39.7; C₁₅H₄O₂ (OH) (OCH₃)₅ requires: OCH₃, 40.0%.]

Alkaline Oxidation of the Pentamethyl Ether: Isolation of 4:5-Dimethyl Gallic Acid.—The pentamethyl cannabiscetin (0.5 g.) was boiled under reflux in a silver flask with 50% potash (20 c.c.) for 6 hours. After cooling, the alkaline solution was diluted, filtered and the clear filtrate acidified with hydrochloric acid. On extraction with ether, the solution yielded a small amount of a crystalline solid which was, however, brown in colour. It was purified by dissolution in sodium bicarbonate and subsequent reprecipitation. On crystallisation from dilute alcohol, the acid was obtained as narrow rectangular plates melting at 193–95°. It was easily soluble in hot water, and the solution did not develop any characteristic colour, when treated with a drop of ferric chloride. The substance was found to be a dimethyl ether of gallic acid. [Found: OCH₃, 31.0; C₆H₂ (OH) (OCH₃)₂ COOH requires OCH₃, 31.3%.] It was not, however, identical with syringic acid (m.p. 204–05°) since the mixed melting point of the two substances was depressed and indefinite, ranging from 155–75°. All the properties indicated that the acid should be 4:5-dimethyl gallic acid, and this was confirmed by taking the mixed melting point of the two substances; it was undepressed. For this purpose an authentic sample of 4:5-dimethyl gallic acid was prepared from vanillin according to the method of Shriner and Mc. Cutchen.⁴ Vanillin was treated with bromine in glacial acetic acid solution, when 3-bromovanillin was produced. It was then methylated with dimethyl sulphate and alkali to yield 3-bromo-4:5-dimethoxy benzaldehyde, which was subsequently oxidised with alkaline potassium permanganate to form the corresponding acid. The bromine atom in this acid was finally replaced by a hydroxyl group by heating it in a sealed tube with dilute alkali and copper powder. 4:5-Dimethyl gallic acid thus obtained melted at 193–95° and not at 184–85° as stated by Shriner and Mc. Cutchan (*loc. cit.*). [Found: OCH₃, 31.2; C₆H₂ (OH) (OCH₃)₂ COOH requires OCH₃, 31.3%.]

Further Methylation of the Pentamethyl Ether: Preparation of Hexamethyl Cannabiscetin.—The pentamethyl cannabiscetin obtained above was dissolved in excess of 20% alkali and methylated with dimethyl sulphate in the usual manner. After the completion of the reaction, the product was acidified with dilute hydrochloric acid, and then extracted with ether. On evaporating off the solvent, a pale yellow solid was obtained. It was purified

by crystallisation from alcohol using a little animal charcoal. The substance was found to be identical with hexamethyl cannabiscetin melting at 174–75°.

Oxidation of Cannabiscitrin: Isolation of 4: 5-Dimethyl Gallic Acid.

—The glucoside (1 g.) was dissolved in 50% potash (15 c.c.) and the blood red solution formed was frequently shaken and exposed to air for 24 hours. It was then diluted to about 40 c.c. and shaken with excess of dimethyl sulphate (15 c.c.) in small quantities. After leaving overnight, the mixture was heated on a water-bath for about half an hour to destroy the excess of dimethyl sulphate. The solution was still alkaline. It was therefore just neutralised with hydrochloric acid, and then the calculated amount of concentrated sulphuric acid was added so as to render the solution 7% in its acid content. It was then boiled under reflux for 2 hours, and when cooled a small amount of resin separated out. This was removed by filtration using a fluted filter, and the clear filtrate was then extracted with ether. On distilling off the ether, a brown viscous liquid was left behind. When it was left overnight in the frigidaire, an almost colourless crystalline solid separated out. It was filtered and recrystallised from boiling water using a little animal charcoal, when it appeared as colourless needles and plates melting at 192–94°. The acid was found to be identical with 4: 5-dimethyl gallic acid.

Summary

The final constitution of cannabiscitrin has been established. It is a monoglucoside of the flavonol cannabiscetin, carrying the sugar group in the side phenyl nucleus in the 3'-position. This has been arrived at from the following considerations:—It gives the gossypetone reaction and after complete methylation and hydrolysis, it yields a pentamethyl cannabiscetin which on decomposition produces 4: 5-dimethyl gallic acid. The same acid is also obtained by first decomposing cannabiscitrin with alkali, and then subjecting the products to methylation and subsequent hydrolysis. Thus the glucoside belongs to an unusual type and resembles butrin.

REFERENCES

1. Neelakantam, Suryaprakasa Rao and Seshadri *Proc. Ind. Acad. Sci.*, 1941, 14, 105.
2. Suryaprakasa Rao and Seshadri *Ibid.*, 1939, 9, 177.
3. ————— .. *Ibid.*, 1941, 14, 29.
4. Shriner and Mc. Cutchan .. *J. A. C. S.*, 1929, 51, 2193.