385. *Glucosides related to Carcinogenic Hydrocarbons.*

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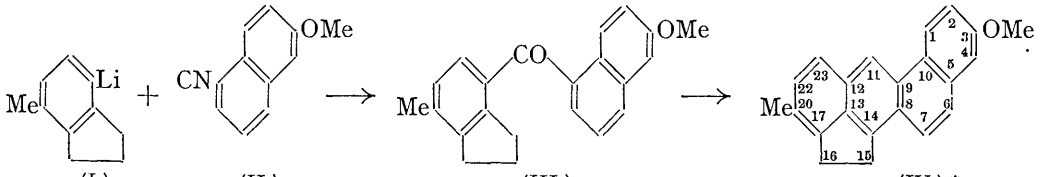
Attempts have been made to synthesise water-soluble cancer-producing compounds which might be expected to have interesting biological properties. Methylcholanthrene is a potent cancer-producing hydrocarbon which can be prepared from the bile acids, and a methylhydroxycholanthrene has been synthesised in which the hydroxyl group occupies the position in the molecule characteristic of the hydroxyl group of many natural sterols. This methylhydroxycholanthrene was converted into its tetra-acetylglucoside, which was hydrolysed to an amorphous glucoside, insoluble in water. In the meantime, it has been found that the methylhydroxycholanthrene has no cancer-producing activity and hence provides an unsuitable basis for the purpose in view. Parallel experiments, with similar results, have been carried out with a hydroxy-derivative of 3 : 4-benzpyrene, a potent cancer-producing hydrocarbon present in coal tar.

Apart from the molecular complexes of carcinogenic hydrocarbons with sodium deoxycholate (Fieser and Newman, *J. Amer. Chem. Soc.*, 1935, **57**, 1602) the only water-soluble carcinogenic compound at present available is sodium 1 : 2 : 5 : 6-dibenzanthracene-9 : 10-*endo*-αβ-succinate (Cook, J., 1931, 3273; Burrows and Cook, *Amer. J. Cancer*, 1936, **27**, 267), the potency of which is rather low, and it seemed possible that suitable water-soluble derivatives of the very active carcinogenic hydrocarbons cholanthrene and 3 : 4-benzpyrene might be more potent. Moreover, the conversion of a carcinogenic hydroxy-compound into a water-soluble derivative (*e.g.*, by combination with a sugar) which would undergo hydrolysis in the animal body might enable one to produce cancer at other sites than that of application of the carcinogenic substance. In the choice of a suitable hydroxy-derivative of cholanthrene for such experiments we were guided by the consideration that, if the transformation of a bile acid into a methylcholanthrene type of molecule happens to occur in the body, the ultimate product might well retain the hydroxyl group at position 3 (sterol system of numbering) characteristic of so many of the natural products of the sterol group.

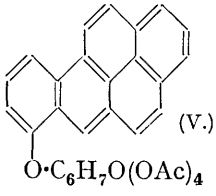
We had a choice of four synthetic methods for the preparation of the cholanthrene ring system, and that due to Fieser and Seligman (*J. Amer. Chem. Soc.*, 1935, **57**, 942)

seemed the most suitable for the desired methylhydroxycholanthrene. The magnesio-derivative of 7-bromo-4-methylhydrindene is very slowly formed, and we found it more convenient to employ 4-methylhydrindyl-7-lithium (I) for interaction with 6-methoxy-1-naphthonitrile (II), a compound which we had already synthesised when it was described by Butenandt and Schramm (*Ber.*, 1935, **68**, 2083). The resulting 7-(6′-*methoxy*-1′-*naphthoyl*)-4-*methylhydrindene* (III) smoothly underwent the Elbs dehydration to 3-*methoxy*-20-*methylcholanthrene* (IV) when heated for 20 minutes at 405°.



(I.) (II.) (III.) (IV.) *

The same modification of the original method of Fieser and Seligman was likewise found preferable for the synthesis of 20-methylcholanthrene, in which case the lithio-compound (I) was condensed with 1-naphthonitrile. The methoxy-compound (IV) was demethylated by hydrobromic acid in boiling acetic acid, giving 3-*hydroxy*-20-*methylcholanthrene*,† which was purified through its acetate. Robertson's procedure (J., 1930, 1136) was followed for the preparation of the glucoside of this phenol, its potassium salt being condensed in aqueous acetone solution with *O*-tetra-acetyl-α-glucosidyl bromide. The resulting 3-O-*tetra-acetyl*-β-*glucosidoxy*-20-*methylcholanthrene* was deacetylated with cold methyl-alcoholic ammonia, but the free glucoside was amorphous and could not be obtained crystalline or analytically pure. It was, however, completely insoluble in water. 4′-O-*Tetra-acetyl*-β-*glucosidoxy*-3 : 4-*benzpyrene* (V) was similarly prepared, and in this case also the free glucoside was amorphous and insoluble in water.



(V.)

In the hope of overcoming this lack of solubility by the introduction of a salt-forming carboxyl group preliminary experiments have been made on the condensation of 4′-hydroxy-3 : 4-benzpyrene with methyl α-triacetylbromo-*d*-galacturonate (Morell, Baur, and Link, *J. Biol. Chem.*, 1935, **110**, 719). The product of this reaction has not yet been obtained analytically pure. Unsuccessful attempts have also been made to prepare the succinic half-esters of 3-hydroxy-20-methylcholanthrene and 4′-hydroxy-3 : 4-benzpyrene.

While these experiments on the production of water-soluble derivatives were in progress the free hydroxy-compounds and also their methyl ethers were submitted to tests for carcinogenic activity, with the somewhat surprising result that all four compounds were found completely devoid of activity (Bachmann *et al.*, *Proc. Roy. Soc.*, 1937, *B*, **123**, 343). The following table, giving the duration of the mouse-painting experiments, supplements the report already published. No tumour has beeen obtained in any mouse.

Compound.	No. of mice.	Duration of experiment (days).
3-Hydroxy-20-methylcholanthrene	10	549
3-Methoxy-20-methylcholanthrene	10	406
4′-Hydroxy-3 : 4-benzpyrene	10	434
4′-Methoxy-3 : 4-benzpyrene	10	444

The lack of carcinogenicity of the hydroxy-compounds defeats the original purpose of transforming them into water-soluble derivatives. Whether the loss of activity is due to the position or to the nature of the hydroxyl group or to both these factors cannot

* The sterol system of numbering used by Fieser and Seligman (*J. Amer. Chem. Soc.*, 1935, **57**, 1377) for denoting the positions of substituents in the cholanthrene molecule; the numbers 18, 19, and 21 are assigned to methyl groups.

† Publication of our work at the present stage is desirable in spite of the fact that the primary object of the investigation has not yet been achieved, because Professor Fieser has very courteously informed us that he and Dr. Riegel have also synthesised this hydroxymethylcholanthrene by substantially the same method that we have employed.

be inferred from the data at present available, but it is certain that the introduction of oxygen does not *necessarily* lead to loss of carcinogenic activity, as is shown by the production of tumours by 9-methoxy-1 : 2 : 5 : 6-dibenzanthracene (Barry *et al.*, *ibid.*, 1935, *B*, **117**, 318) and by 3-hydroxy-1 : 2-benzanthracene and its methyl ether (Fieser, Hershberg, Long, and Newman, *J. Amer. Chem. Soc.*, 1937, **59**, 475). Hence we propose to continue our search for potent carcinogenic hydroxy-compounds which may serve as a basis for the production of water-soluble derivatives.

The ultra-violet absorption spectra of 3-hydroxy-20-methylcholanthrene and its methyl ether have been determined by Dr. E. Roe, and are in accord with the structures of substituted 1 : 2-benzanthracenes assigned to these compounds.

Experimental.

6-Methoxy-1-naphthonitrile (II).—In the preparation of this compound from the corresponding diazonium salt better yields were obtained with nickel cyanide than with cuprous cyanide, the procedure being based on that of McRae (*J. Amer. Chem. Soc.*, 1930, **52**, 4550), adapted from the method of Korczynski and Fandrich (*Compt. rend.*, 1926, **183**, 421).

A suspension of 6-methoxy-1-naphthylamine sulphate (Cohen, Cook, and Hewett, J., 1934, 656; 1935, 451) (15 g.) in water (200 c.c.) and concentrated hydrochloric acid (30 c.c.) was diazotised at 0° with sodium nitrite (4 g. in 60 c.c. of water). The resulting solution was added during ½ hour to a well-stirred ice-cold solution of nickel cyanide (75 c.c.) covered with a layer of benzene. The cyanide solution was prepared by addition of a solution of pure nickel nitrate (36·5 g.) in water (60 c.c.) to a solution of potassium cyanide (40·7 g.) and sodium hydroxide (10 g.) in water (140 c.c.). The mixture was stirred at 0° for a further ½ hour, then allowed to warm to room temperature, and finally, after 1½ hours, heated to 50° and maintained at this temperature for ½ hour. The liquid was filtered, and the tarry residue extracted several times with warm benzene. The extract was dried and distilled; the 6-methoxy-1-naphthonitrile (b. p. 154°/0·3 mm.) crystallised from ligroin in colourless prisms (35% yield), m. p. 78—79°, in agreement with Butenandt and Schramm (*loc. cit.*) (Found : C, 78·9; H, 5·1. Calc. : C, 78·7; H, 4·9%).

7-(6′-Methoxy-1′-naphthoyl)-4-methylhydrindene (III).—A solution of 7-bromo-4-methylhydrindene (Fieser and Seligman, *J. Amer. Chem. Soc.*, 1935, **57**, 942) (4·2 g.) in anhydrous ether (30 c.c.) was added during an hour, with repeated shaking, to a suspension of powdered lithium (0·4 g.) in anhydrous ether (25 c.c.) kept under nitrogen. Reaction was completed by 6 hours' boiling, and a solution of 6-methoxy-1-naphthonitrile (3·6 g.) in ether (25 c.c.) and benzene (15 c.c.) was slowly added to the ice-cold solution of the lithium compound (I). The whole was boiled for 24 hours, and the product decomposed with ice. In order to hydrolyse the ketimine primarily formed in this reaction the material from the ethereal layer was refluxed for 2 hours with 10% hydrochloric acid, and re-extracted with ether and distilled. The *ketone* (III) (3·1 g., b. p. 245—250°/1 mm.) crystallised from alcohol in large colourless prisms, m. p. 86—87° (Found : C, 83·5; H, 6·4. $C_{22}H_{20}O_2$ requires C, 83·4; H, 6·3%).

3-Methoxy-20-methylcholanthrene (IV).—The aforesaid ketone (3 g.) was heated at 405° for 20 minutes in an atmosphere of carbon dioxide, and the residue distilled in a vacuum (b. p. 240—245°/0·3 mm.). The orange crystalline distillate (2·3 g.) was treated, in benzene solution, with picric acid, and gave purplish-black prisms of the picrate of 3-methoxy-20-methylcholanthrene, m. p. 178—179°. This picrate was freed from picric acid by shaking with ether and aqueous ammonia. The resulting *3-methoxy-20-methylcholanthrene* (IV) (1 g.) crystallised from benzene–alcohol in light straw-coloured needles, m. p. 165—166·5° (corr.) (Found : C, 88·7; H, 6·2; OMe, 9·8. $C_{22}H_{18}O$ requires C, 88·55; H, 6·1; OMe, 10·4%).

3-Hydroxy-20-methylcholanthrene.—For demethylation, a suspension of the methoxy-compound (IV) (1·65 g.) in glacial acetic acid (40 c.c.) and hydrobromic acid (*d* 1·48; 17 c.c.) was boiled for 3 hours, during which a clear solution was obtained. The product was precipitated with water, collected, recrystallised from glacial acetic acid (charcoal), and then acetylated by warming for 5 minutes with acetic anhydride (2 c.c.) in pyridine (4 c.c.). *3-Acetoxy-20-methylcholanthrene* crystallised from ethyl acetate in straw-coloured rhombs, m. p. 191—192° (corr.) (Found: C, 84·9; H, 5·7. $C_{23}H_{18}O_2$ requires C, 84·6; H, 5·6%). For hydrolysis a suspension of this acetate (0·75 g.) in alcohol (40 c.c.) and 50% aqueous potassium hydroxide (1 c.c.) was boiled for 3 minutes in an atmosphere of nitrogen. The orange solution was diluted with water and acidified with hydrochloric acid, and the

precipitate dried and recrystallised from methyl alcohol–benzene. 3-*Hydroxy*-20-*methylcholanthrene* (0·45 g.) formed golden-yellow leaflets, m. p. 218·5—220° (corr.) in a bath preheated to 200° (Found : C, 88·4; H, 5·8. $C_{21}H_{16}O$ requires C, 88·7; H, 5·7%).

3-O-*Tetra-acetyl*-β-*glucosidoxy*-20-*methylcholanthrene*.—*O*-Tetra-acetyl-α-glucosidyl bromide (0·4 g.) was added to a solution of 3-hydroxy-20-methylcholanthrene (0·3 g.) in acetone (10 c.c.) and 12% aqueous potassium hydroxide (2 c.c.). Further quantities of the bromide (0·4 g.) and 12% potash (2 c.c.) were added after 16 hours at room temperature, and the whole kept for another 2 days. The *tetra-acetyl glucoside* was collected and recrystallised from alcohol, forming pale yellow needles, m. p. 210—211° (Found : C, 67·9; H, 5·6. $C_{35}H_{34}O_{10}$ requires C, 68·4; H, 5·6%).

4′-O-*Tetra-acetyl*-β-*glucosidoxy*-3 : 4-*benzpyrene* (V).—The 4′-hydroxy-3 : 4-benzpyrene was prepared by sulphur-dehydrogenation of 4′-keto-1′ : 2′ : 3′ : 4′-tetrahydro-3 : 4-benzpyrene (Fieser, Hershberg, and Newman, *J. Amer. Chem. Soc.*, 1935, **57**, 1509) and purified through its acetate. The hydroxy-compound (1 g.) in acetone (15 c.c.) and water (5 c.c.) was twice treated with potassium hydroxide (0·2 g.) and *O*-tetra-acetyl-α-glucosidyl bromide (1·5 g.), with an interval of 24 hours. After a second 24 hours the pale yellow silky needles of the *tetra-acetyl glucoside* (V) were collected and recrystallised from alcohol (yield, 0·4 g.); they then had constant m. p. 184—185° (Found : C, 68·3; H, 5·2. $C_{34}H_{30}O_{10}$ requires C, 68·2; H, 5·1%). For deacetylation to the free glucoside a suspension of this tetra-acetate in saturated methyl-alcoholic ammonia was kept in the ice-chest for 36 hours. The flocculent precipitate was collected and dissolved in hot alcohol, and the solution cooled. The glucoside, not obtained pure, separated as a gelatinous precipitate which dried to a greenish-yellow powder, m. p. 270—273° (Found : C, 70·5; H, 5·7. $C_{26}H_{22}O_6$ requires C, 72·5; H, 5·2%).

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