THE SYNTHESIS OF TWO ISOMERIC α-HYDROJUGLONE GLUCOSIDES

By C. DAGLISH

From the Ovaltine Research Laboratories, King's Langley, Herts.

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ATTEMPTS to synthesise α-hydrojuglone-5-β-D glucoside isolated[1] from the walnut (*Juglans regia*) and shown[2] to be responsible for the "apparent vitamin C" activity,[3] gave rise to two isomeric tetra-acetylated glucosides[4] which could be further acetylated, apparently, to the same hexa-acetate. It was assumed that these compounds were the 5-acetylated glucosides of α- and β-hydrojuglones, because both these isomers had proved suitable starting material. This work has been expanded, and it would now appear that whereas the first compound is α-hydrojuglone-5-tetra-acetyl-β-D glucoside, which by hydrolysis of its acetyl groups becomes identical with the compound isolated from the walnut, the second has the glucose moiety attached to the naphthalene nucleus through the hydroxyl in the 1 position. Details of the work leading to these conclusions are given below.

EXPERIMENTAL

Tetra-acetyl-α-D-glucosyl bromide was prepared from glucose penta-acetate[5] in 84·5 per cent. yield.

α-Hydrojuglone (1:4:5-trihydroxynaphthalene) was prepared from juglone by the method of Willstatter and Wheeler.[6] The ethereal solution (of α-hydrojuglone) was filtered through a column of dried magnesium sulphate into light petroleum (b.pt. 60° to 80° C.) and the α-hydrojuglone rapidly separated as colourless needles m.pt. 148° C. Yield 89·7 per cent.

β-Hydrojuglone (5-hydroxy-2:3-dihydro-1:4-naphthoquinone) was prepared by vacuum distillation of α-hydrojuglone from an oil bath at 170° C. and recrystallising the distillate from ethanol to give pale yellow needles m.pt. 96° to 97° C. Yield (from juglone) 65 per cent.

Tetra-acetylated glucosides. (*a*) Juglone 6·96 g. (0·04 mole) and tetra-acetylglucosyl bromide 16·44 g. (0·04 mole) were dissolved in dry acetone 600 ml. 1·5N ethanolic potassium hydroxide 26·5 ml. (0·04 mole) was added under nitrogen, then the flask was stoppered and left for 16 hours at room temperature in the dark. The dark violet reaction mixture was filtered, the filtrate was evaporated to dryness under reduced pressure and the residue extracted with ethanol, 600 ml. The extract showed (spectrophotometrically) the presence of 1·06 g. of α-hydrojuglone-5-tetra-acetyl-β-D glucoside (5·25 per cent. of theory). This could not be separated from the tarry side-reaction products by the normal methods of crystallisation, so the extract was chromatographed on alumina in the manner described for the natural glucoside.[1] This gave 0·84 g. (4·2 per cent.) of α-hydrojuglone-5-tetra-acetyl-β-D glucoside.

(*b*) α-Hydrojuglone 5·27 g. (0·03 mole) and tetra-acetylglucosyl bromide 12·33 g. (0·03 mole) were dissolved in acetone 70 ml. and 1·5N

ethanolic potassium hydroxide 20 ml. (0·03 mole) added under nitrogen. After 16 hours, glacial acetic acid 2 ml. was added and the mixture filtered from the sandy precipitate of potassium bromide which had first separated after 15 minutes. The filtrate was evaporated to dryness under reduced pressure and the yellow-brown crumbly residue stirred with ether 20, 10 and 10 ml., transferred to a filter and sucked dry. The almost white residue was stirred with ethanol (95 per cent.) 20 ml. refiltered and washed with a further 10 ml. This gave 7·78 g. of mixed tetra-acetylated glucosides. Taken up in boiling ethanol 75 ml. α-hydrojuglone-1-tetra-acetyl-β-D glucoside crystallised immediately on cooling (6·34 g.).

The mother liquors, together with the ethereal and ethanolic washes, were evaporated to dryness under reduced pressure. The residue, extracted with chloroform, precipitated with light petroleum (b.pt. 60° to 80° C.) and recrystallised from benzene, gave α-hydrojuglone-5-tetra-acetyl-β-D glucoside 3·59 g. Overall yield 65·4 per cent.

(*c*) β-Hydrojuglone 5·28 g. (0·03 mole) treated in the same manner gave α-hydrojuglone-1-tetra-acetyl-β-D glucoside 5·27 g. and α-hydrojuglone-5-tetra-acetyl-β-D glucoside 3·00 g. Overall yield 54·6 per cent.

α-Hydrojuglone-5-tetra-acetyl-β-D-glucoside. Readily soluble in ethanol, acetone, ether, chloroform, ethyl acetate, benzene and acetic acid; less so in light petroleum and insoluble in water; it crystallised from benzene as white microcrystals m.pt. 179° C. (decomp.). Found: C, 55·8; H, 5·79; CH_3CO, 33·7 per cent.; $C_{24}H_{26}O_{12}$ requires, C, 56·9; H, 5·14; CH_3CO, 34·0 per cent. It slowly decomposed in ethanol solution but was quite stable in acid ethanol, in which it showed (Fig. 1)

$\lambda_{max.}$	*225*	*308*	*326*	*341*	mμ.
$E^{1\text{ per cent.}}_{1\text{ cm.}}$	791	123·3	122·3	117·6	

and $[\alpha]_D^{20°\text{ C.}}$ — 53·5° C. 0·04 per cent.

1·15 g. was refluxed for 10 minutes with acetic anhydride 9 ml. and anhydrous sodium acetate 1 g., the mixture was cooled and diluted with water and the precipitated solid recrystallised from ethanol then ether, gave the hexa-acetate of α-hydrojuglone-5-β-D glucoside, 0·85 g. (63·5 per cent.) as tiny white needles m.pt. 140° to 141° C. Found: C, 56·9; H, 5·22; CH_3CO, 41·0 per cent. $C_{28}H_{30}O_{14}$ requires, C, 57·0; H, 5·08; CH_3CO, 43·7 per cent.

0·25 g. in methanol, 10 ml., treated with 3 per cent. w/v ethereal diazomethane solution, 20 ml., for 3 hours, gave on evaporation and recrystallisation of the residue from methanol 4 ml., 0·177 g. of 1:4-dimethoxy-5-hydroxynaphthalene-5-tetra-acetyl-β-D glucoside m.pt. 174° to 175° C. Found: C, 58·2; H, 5·58; CH_3O, 11·65 per cent. $C_{26}H_{30}O_{12}$ requires, C, 58·4; H, 5·6; CH_3O, 11·6 per cent.

The dimethoxy derivative, 0·15 g., in methanol, 10 ml., refluxed for 30 minutes with 2N hydrochloric acid, 7·5 ml., gave 5-hydroxy-1:4-dimethoxynaphthalene 0·03 g. (55 per cent.) m.pt. 155° to 156° C. The melting point was not depressed by admixture with an authentic sample prepared from juglone acetate by the method of Ruelius and Gauhe.[7] Found: C, 69·7; H, 5·85 per cent. $C_{12}H_{12}O_3$ requires C, 70·6; H, 5·88 per cent.

α-Hydrojuglone-5-β-D-glucoside. (*a*) A solution of α-hydrojuglone-5-tetra-acetyl-β-D glucoside, 1 g., in methanol, 25 ml., from which the air had been displaced by a stream of nitrogen, was treated for 1 hour with 2N ethanolic potassium hydroxide, 2 ml. The colour became deep purple but this was discharged on adding 2N sulphuric acid, 2 ml. The mixture was

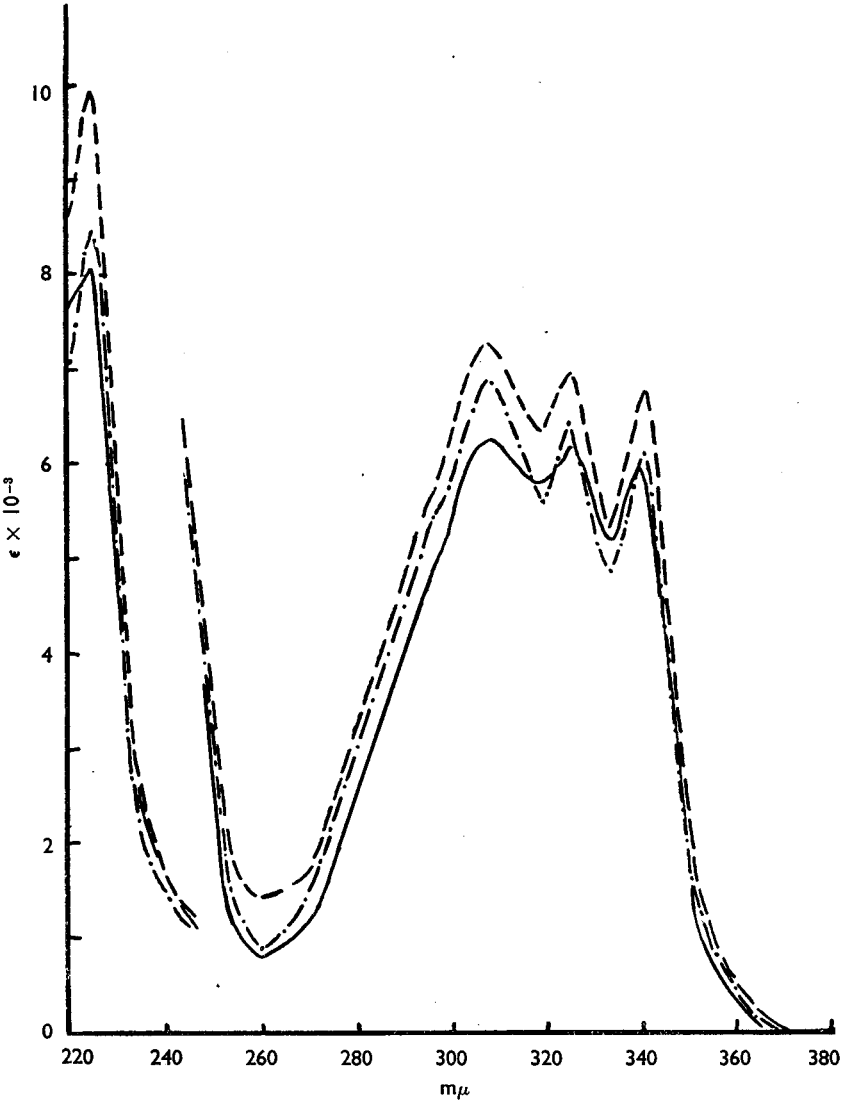


FIG. 1. Absorption curves in 0·1N ethanolic hydrochloric acid of
——— α-hydrojuglone-5-tetra-acetyl-β-D glucoside.
– – – α-hydrojuglone-5-β-D glucoside, synthetic.
– · – · – · α-hydrojuglone-5-β-D glucoside, natural.
Left-hand curve is 1–5 dilution of that on right.

centrifuged to remove precipitated potassium sulphate, and the supernatant liquid diluted with water, 25 ml., and evaporated at reduced pressure till all the methanol had been removed. The aqueous residue was extracted with ethyl acetate to give α-hydrojuglone-5-β-D glucoside 0·32 g. (48·2 per cent.).

(*b*) A solution of the hexa-acetate of α-hydrojuglone-5-β-D glucoside, 1·59 g., in methanol, 25 ml., was hydrolysed in the same manner, giving 0·55 g. (59 per cent.) of α-hydrojuglone-5-β-D glucoside.

The glucoside was a light fawn coloured microcrystalline powder. Found: C, 57·7; H, 5·87 per cent. $C_{16}H_{18}O_8$ requires C, 56·8; H, 5·32 per cent. The melting point 178° C. (decomp.) was not depressed by admixture with the glucoside isolated from the walnut.

α-Hydrojuglone-1-tetra-acetyl-β-D glucoside. Readily soluble in chloroform, acetone, ethyl acetate and acetic acid; less so in ether, ethanol and light petroleum; it crystallised from ethanol as white needles m.pt. 196° to 197° C. Found: C, 56·5; H, 5·14; CH_3CO, 33·1 per cent. $C_{24}H_{26}O_{12}$ requires C, 56·9; H, 5·14; CH_3CO, 34·0 per cent. Solutions in acid ethanol were quite stable and showed (Fig. 2)

$\lambda_{max.}$	*238*	*315*	*333*	*343*	mμ
$E^{1\text{ per cent.}}_{1\text{ cm.}}$	337	130	157·4	155·2	

and $[\alpha]_D^{20°\text{ C.}}$ — 75·5° C. 0·1 per cent.

1 g. refluxed for 10 minutes with acetic anhydride, 5 ml., and anhydrous sodium acetate, 1 g., cooled, added to water, 70 ml., and the precipitated solid recrystallised from ethanol then ether, gave the hexa-acetate 0·8 g. (68·5 per cent.) m.pt. 140° to 141° C. as tiny white needles. Found: C, 57·2; H, 5·3; CH_3CO, 43 per cent. $C_{28}H_{30}O_{14}$ requires C, 57·0; H, 5·1; CH_3CO, 43·7 per cent. The melting point did not appear to be depressed by admixture with the hexa-acetate prepared from the natural glucoside nor with that from α-hydrojuglone-5-tetra-acetyl-β-D glucoside, and like these it showed in acid ethanol

$\lambda_{max.}$	*226*	*288*	*295*	*325*	mμ
$E^{1\text{ per cent.}}_{1\text{ cm.}}$	1092	126·8	144	41·8	

$[\alpha]_D^{20°\text{ C.}}$ — 83·7° C. 1 per cent.

0·5 g. in methanol, 40 ml., treated with 3 per cent. w/v ethereal diazomethane solution, 50 ml., for 1 hour at room temperature, the solvent removed at the pump and the residue recrystallised from 4 ml. of methanol, gave the monomethoxy derivative, 0·222 g., as microneedles, m.pt. 148° to 149° C. Found: C, 57·3; H, 5·3; CH_3O, 6·26 per cent. $C_{25}H_{28}O_{12}$ requires C, 57·7; H, 5·38; CH_3O, 6·0 per cent.

α-Hydrojuglone-1-β-D glucoside. (*a*) A solution of α-hydrojuglone-1-tetra-acetyl-β-D glucoside, 1 g., in methanol, 25 ml., was hydrolysed for 1 hour at room temperature with 2N ethanolic potassium hydroxide, 2 ml., under nitrogen and the free glucoside extracted with ethyl acetate as described above. Yield 0·5 g. (75 per cent.) m.pt. 179° to 180° C.

(*b*) The hexa-acetate of α-hydrojuglone-1-β-D glucoside, 1·9 g., in

methanol, 25 ml., treated in the same manner gave 0·77 g. of glucoside (70·3 per cent.) m.pt. 179° to 180° C. Readily soluble in water, ethanol and acetone, less so in ethyl acetate and *iso*amyl alcohol and insoluble in ether, chloroform and light petroleum, it crystallised from ethyl acetate as white microcrystals, m.pt. 179° to 180° C. Found: C, 55·5; H, 5·41

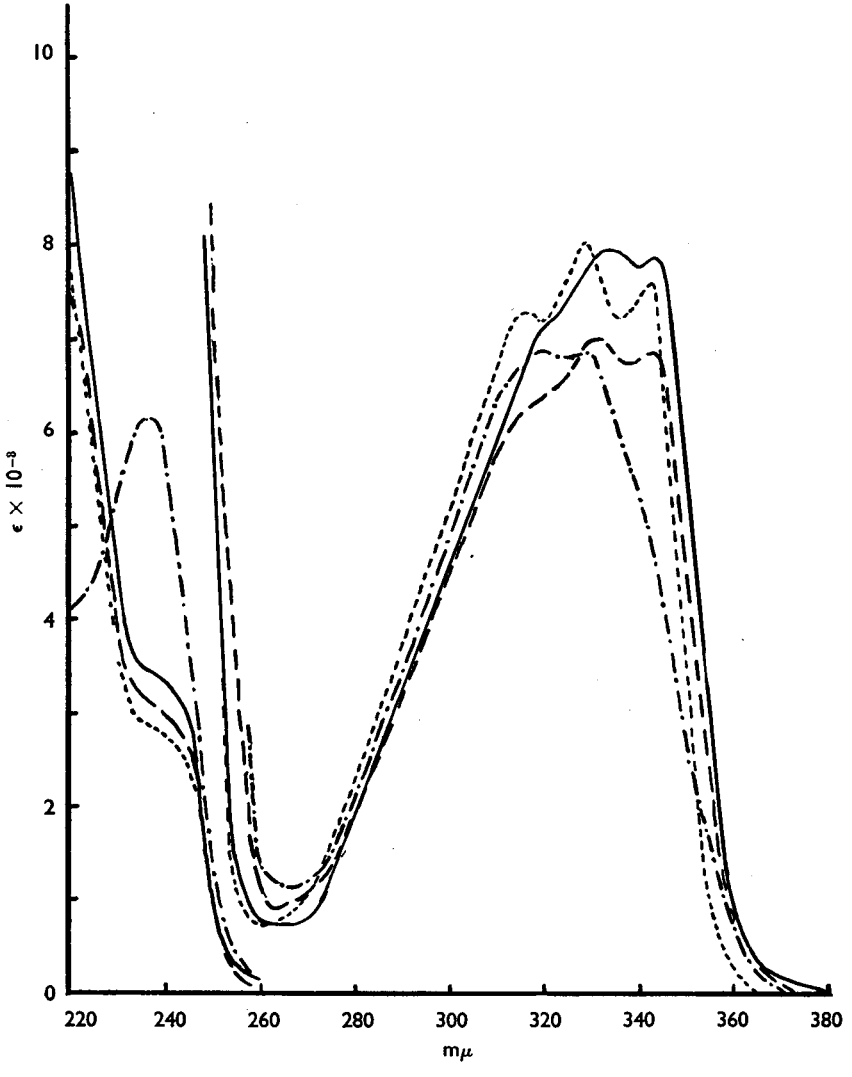


FIG. 2. Absorption curves in 0·1 N ethanolic hydrochloric acid.

———— α-hydrojuglone-1-tetra-acetyl-β-D glucoside.
— — — α-hydrojuglone-1-β-D glucoside.
– – – – – α-hydrojuglone-1-β-D glucoside methoxy derivative.
– · – · – · 1 : 8-dihydroxynaphthalene-4-sulphonic acid.

Left-hand curve is 1–5 dilution of that on right.

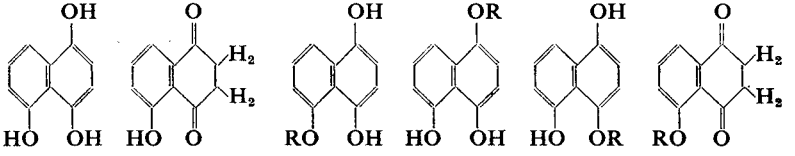
per cent. $C_{16}H_{18}O_8$ requires C, 56·8; H, 5·32 per cent. Ethanolic solutions were unstable, but in acid ethanol it showed (Fig. 2)

$\lambda_{max.}$	*240*	*320*	*333*	*344*
$E^{1\text{ per cent.}}_{1\text{ cm.}}$	444	187·8	207·5	203·5

and $[\alpha]_D^{20°\text{ C.}}$ — 93° C. 0·1 per cent.

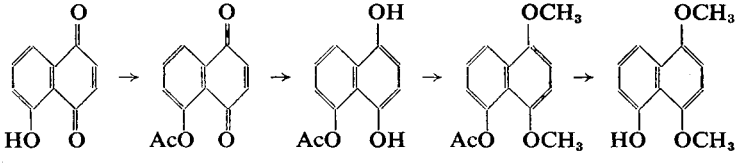
Discussion

The formation of two acetylated glucosides in a constant proportion of 1:1·75 from both α- and β-hydrojuglone (I and II) by the action of tetra-acetylglucosyl bromide in the presence of one equivalent of potassium hydroxide, suggests that one of these has structure III, whilst the other is IV, V or VI.



where R is $C_{14}H_{19}O_9$.

The isomer more readily soluble in ether, on hydrolysis of the acetyl groups gives rise to a glucoside identical with that found in acid ethanol extracts of the walnut. That this has structure III is shown by its reaction with diazomethane to give a dimethoxy derivative, which on heating with acid is hydrolysed to 5-hydroxy-1:4-dimethoxynaphthalene. This latter compound has been synthesised by the method of Ruelius and Gauhe[7] as follows



It has been suggested[4] that the second tetra-acetylated glucoside has structure VI. The evidence for this was based upon the facts that it was readily prepared from β-hydrojuglone, and that its hexa-acetate appeared in melting point and ultra-violet absorption data (Fig. 3) identical with that obtained from III. It showed, however, no ketonic properties, and attempts to prepare derivatives with *p*-nitrophenylhydrazine, phenylhydrazine and phenylsemicarbazide resulted only in the formation of coloured solutions from which the bulk of the glucoside was recovered unchanged. Infra-red examination of this compound revealed that instead of the carbonyl groups expected, there was absorption due to two hydrogen bonded hydroxyl groups.

Of the alternative structures IV and V, the former, with a *peri*naphthalene structure would be expected to show some hydrogen bonding, whereas the latter would not, unless the glucose portion of the molecule

were involved. It is probably due to this bonding that with diazomethane only a monomethoxy derivative is formed by the glucoside. Since the same result is obtained with 1:8-dihydroxynaphthalene[8,9] it appears that IV is the more likely structure for the second glucoside. Some further slight evidence for this is shown (Fig. 2) in the similarity of its ultra-violet absorption curve with that of 1:8-dihydroxynaphthalene-4-sulphonic acid.

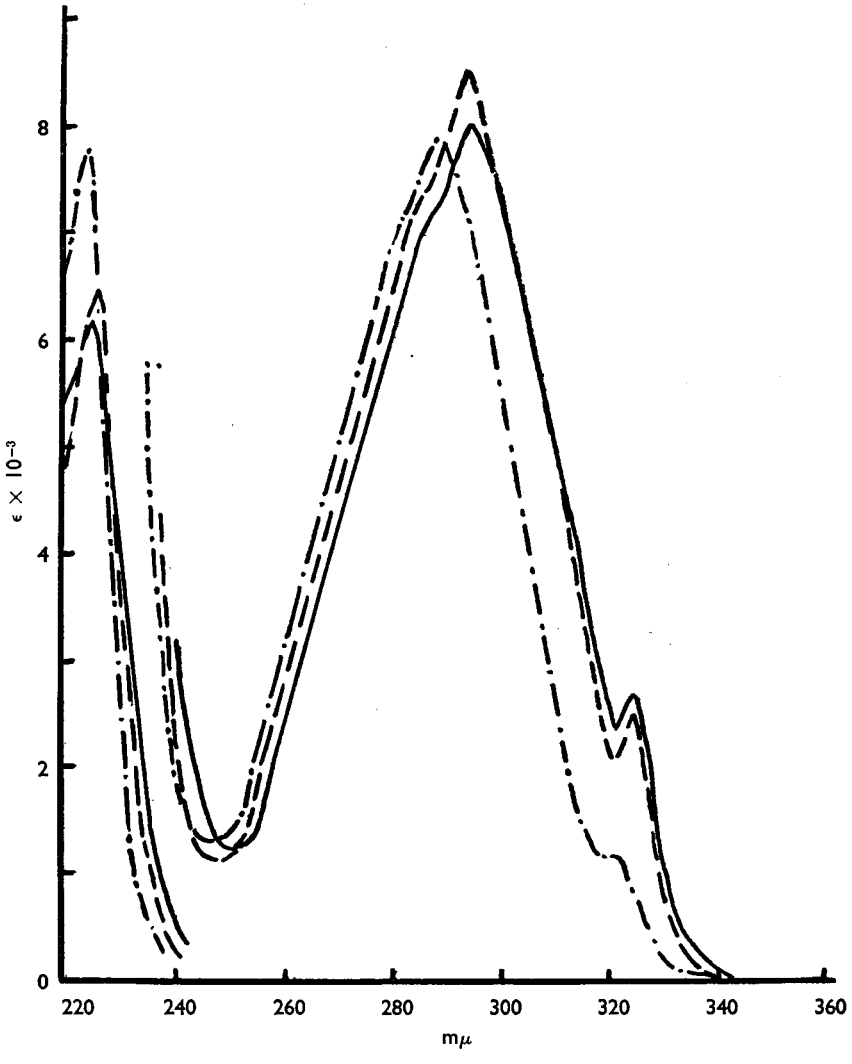


FIG. 3. Absorption curves in 0·1N ethanolic hydrochloric acid of
——— Hexa-acetate of α-hydrojuglone-5-β-D glucoside.
– – – – Hexa-acetate of α-hydrojuglone-1-β-D glucoside.
–·–·–· 1:4:5-triacetoxynaphthalene.
Left-hand curve is 1–10 dilution of that on right.

SUMMARY

1. Two tetra-acetylated glucosides have been prepared in 55 to 65 per cent. yield from both α- and β-hydrojuglone.

2. Removal of the acetyl groups from the more ether-soluble of these gives α-hydrojuglone-5-β-D glucoside identical with that isolated from the walnut.

3. It has been shown that the second glucoside is not β-hydrojuglone-5-β-D glucoside as previously reported, but probably α-hydrojuglone-1-β-D glucoside.

I wish to thank Dr. F. Wokes, the Director of the Laboratories, for affording the opportunity to publish this work (part of which was included in the thesis for a Ph.D. degree of the University of London), Miss Nora Baxter for technical help and Dr. R. H. Thomson of Aberdeen for a gift of 1 : 8-dihydroxynaphthalene-4-sulphonic acid. Carbon, hydrogen, acetyl and methoxyl determinations were made by Drs. Weiler and Strauss of Oxford.

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