Anthoxanthins. Part I. Selective Methylation and Demethylation.

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The relative rates of cleavage of methoxyflavones by hydrobromic acid lie in the order 3′ > 4′ > 7. Hydrobromic acid caused selective demethylation of 7 : 3′-dimethoxy-, 3 : 7 : 3′-trimethoxy-, and 5 : 7 : 3′-trimethoxyflavones to yield the 7-monomethyl ethers.

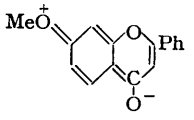
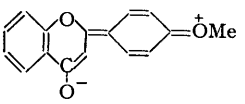
The relative rates of methylation of flavone-hydroxyl groups with methyl sulphate and sodium hydrogen carbonate in acetone lie in the order 7 > 4′ > 3′ > 3. With methyl sulphate and aqueous-alcoholic sodium carbonate the exact reverse of this order is observed. By these methods, selective methylation of a number of hydroxyflavones has been achieved, *e.g.*, galangin yielded its 7-methyl ether by the first method and its 3-methyl ether by the second.

ANTHOXANTHINS are important substances in foodstuffs and their mode of formation in plant tissue is still incompletely known. Stephens (*Arch. Biochem.*, 1948, **18**, 457) has advanced genetical evidence which suggests that glycosidation is the last stage of flavone biosynthesis. In a recent review (*Ann. Rev. Biochem.*, 1951, **20**, 506), Seshadri has pointed out that, although 7-methyl ethers and glycosides are of frequent natural occurrence, attempts to methylate selectively the 7-hydroxyl group in polyhydroxyflavones have failed. He therefore concluded that such a methylation or glycosidation is unlikely to be successful in the plant and tentatively suggested that they take place at a flavanone stage of synthesis. The fact that in the flavonol series the 3-hydroxyl group appears to be favoured for glycosidation has not been discussed. The work described in this communication was undertaken in order to provide information on the relative reactivities of hydroxyl and methoxyl groups occupying different positions in the flavone nucleus.

The rates of acidic fission of 7-, 4′-, and 3′-methoxyflavones have been measured, excess of aqueous hydrobromic acid at 123° being used as reagent (see p. 4066). For demethylation of phenol ethers under similar conditions Donnan and Ghaswalla (*J.*, 1936, 1341) proposed the mechanism :

$$PhOMe \rightleftharpoons H_2O + Ph\text{–}\overset{+}{O}(H)\text{–}Me;\quad Ph\text{–}\overset{+}{O}(H)\text{–}Me + Br^- \longrightarrow PhOH + MeBr$$

The slower cleavage of conjugated than of unconjugated ethers is now explained by quinonoid resonance contributions opposing the approach of hydroxonium ions. In agreement with this, we have found that the 3′-methyl ethers of flavones undergo fission much more readily than the 7- or the 4′-methyl ethers. The observation that the 7-ether is more resistant than the 4′-ether is also to be expected, since the form (I), with its smaller

(I)   (II)

separation of ionic charges must be expected to make a larger contribution to resonance structure than does (II).

The cleavage of polymethoxyflavones by hydrobromic acid has been investigated. 7 : 3′-Dimethoxyflavone was found to undergo selective demethylation, yielding 3′-hydroxy-7-methoxyflavone. 3 : 7 : 3′- and 5 : 7 : 3′-Trimethoxyflavones suffered the expected simultaneous fission of 3′- and 3- or 5-methoxyl groups to give the corresponding 7-monomethyl ethers.

An attempt to achieve the selective methylation of 7 : 4′-dihydroxyflavone with methyl sulphate and potassium carbonate in acetone failed, the product being largely dimethyl ether and unchanged starting material. When the reaction was repeated, with sodium hydrogen carbonate in place of potassium carbonate, the product contained a monomethyl

ether fraction only slightly contaminated with dimethyl ether and starting material : comparison of the fluorescence of this material in ultra-violet light with the fluorescence colours of standard mixtures of 7- and 4′-monomethyl ethers suggested a predominance of the former compound in the product, and this was supported by the isolation of 4′-hydroxy-7-methoxyflavone.

In a more detailed study acetone solutions of hydroxyflavones were allowed to react with excess of methyl sulphate and sodium hydrogen carbonate at 50°, in an atmosphere of carbon dioxide, and at intervals samples were analysed by a gravimetric method. First-order rate constants are given below. Although the precise mechanism of this heterogeneous reaction has not been elucidated, the order of reactivity of flavone-hydroxyl groups ($7 > 4' > 3' > 3$) suggests that the reaction rate is determined by the acidity of the hydroxyl group, an increase in acidity causing an increase in rate.

In a second series of experiments, the reactions between hydroxyflavones and excess of methyl sulphate in aqueous-alcoholic sodium carbonate were followed by spectrophotometric estimation of the phenoxide ions. The rate constants (see p. 4067) fall into the order $3 > 3' > 4' > 7$, in substantial agreement with the view of Hodgson and Nixon (*J.*, 1930, 2166) that the reaction is controlled by the basic strength of the phenoxide ions, a decrease in rate following a decrease in basic strength.

These two, distinct, methylation methods achieve selective methylation of a number of di- and tri-hydroxyflavones; by the first procedure, 3 : 7- and 7 : 3′-dihydroxyflavone yielded the 7-monomethyl ethers, 3 : 7 : 3′-trihydroxyflavone yielded 3 : 3′-dihydroxy-7-methoxyflavone, and galangin (3 : 5 : 7-trihydroxyflavone) yielded its naturally occurring 7-methyl ether, izalpinin; by the second procedure 3 : 7- and 7 : 3′-dihydroxyflavone furnished the 3- and the 3′-monomethyl ether, respectively. In the same way, galangin yielded its naturally occurring 3-methyl ether, and 3 : 4′-dihydroxyflavone gave 4′-hydroxy-3-methoxyflavone, though in unsatisfactory yield. These products were compared with authentic specimens. The new compounds were also synthesised by well-established methods.

It is interesting that the two hydroxyl groups, *viz.*, those occupying the 7- and the 3-position, which are most readily methylated by the methods described above, are those which are apparently favoured for glycosidation in plants. The argument against glycosidation being the last stage of flavone biosynthesis (Seshadri, *loc. cit.*) is therefore no longer valid.

Experimental

M. p.s were measured on a Kofler block and are corrected.

Demethylation Kinetics.—Centrifuge tubes containing solutions of the hydroxyflavones (10 mg.) in hydrobromic acid (48% w/w; 1 ml.) were placed in an oil-bath at 123°. At appropriate intervals, the mixtures were diluted with water (10 ml.) and centrifuged. The supernatant liquors were removed by a capillary siphon, and the solids extracted at the centrifuge with ice-cold 2N-sodium hydroxide (3 × 5 ml.) and then with water (5 ml.). The residues consisting of unchanged ether were transferred to tared, medium-porosity filter tubes, washed with water, dried, and weighed. Pseudo-first-order rate constants were :

Methoxyflavone	7-	4′-	3′-
10^3k_1 (min.$^{-1}$)	8·7	30·5	160

Methylation Kinetics.—(*a*) Mixtures of the appropriate hydroxyflavone (476 mg.), anhydrous acetone (100 ml.), methyl sulphate (2·52 g. 10 mols.), and anhydrous sodium hydrogen carbonate (10 g.) were stirred at 50° under reflux in a slow stream of anhydrous carbon dioxide. At intervals, 3 ml. portions were transferred to centrifuge tubes and diluted with water (20 ml.), and the methyl ethers were separated and determined gravimetrically as in the preceding experiment. Rate constants were :

Hydroxyflavone	7-	4′-	3′-	3-
10^5k_1 (min.$^{-1}$)	110	37	9·8	5·3

(*b*) Methyl sulphate (0·5-ml. portions) was rapidly added, with shaking, to solutions of the hydroxyflavones (20 mg.) in alcohol (3 ml.) and aqueous sodium carbonate (N, 5 ml.), and the solutions were replaced in a water-bath at 20°. At suitable times, 1-ml. portions were removed and diluted with an appropriate volume of aqueous-alcoholic sodium hydroxide (alcohol, 1 vol.;

2N-aqueous sodium hydroxide, 1 vol.). The intensity of colour due to unchanged phenoxide ions was then measured with a " Spekker " absorptiometer. Control experiments showed that Beer's law was obeyed over the absorption range encountered and that absorption by flavone ethers was negligible. The following rate constants for the methylation reaction have been calculated :

Hydroxyflavone	7-	4'-	3'-	3
10^2k_1 (min.$^{-1}$)	1·1	4·3	11·7	17·1

Selective Demethylation of Flavone Ethers.—(*a*) A solution of 7 : 3'-dimethoxyflavone (250 mg.) in hydrobromic acid (48% w/w; 50 ml.) and acetic acid (18·5 ml.) was refluxed gently for 1 hr., cooled, and added to excess of dilute sulphurous acid. The solid was collected and after being washed with a little alcohol had m. p. 244—247°. A chromatogram on Whatman No. 1 paper with benzene–pyridine–water (Simpson and Garden, *J.*, 1952, 4368) showed this material to be 3'-hydroxy-7-methoxyflavone contaminated with a trace of dihydroxy-compound. One recrystallisation from alcohol furnished the pure monomethyl ether in colourless needles (100 mg.), m. p. and mixed m. p. 249—250°.

(*b*) 3 : 7 : 3'-*Trimethoxyflavone* was obtained by methylation of 7-hydroxy-3 : 3'-dimethoxyflavone (Shaw and Simpson, *J.*, 1952, 5031) with potassium carbonate and excess of methyl sulphate in boiling acetone. It separated from light petroleum (b. p. 100—120°) in colourless felted needles, m. p. 122—123° [Found : C, 69·0; H, 5·1; OMe, 29·9. $C_{15}H_7O_2(OMe)_3$ requires C, 69·2; H, 5·2; OMe 29·8%]. A solution of this ether (250 mg.) in hydrobromic acid (48% w/w; 20 ml.) was refluxed gently for 20 min. and then added to water (100 ml.). The precipitate was collected and twice recrystallised from alcohol, yielding 3 : 3'-dihydroxy-7-methoxyflavone in cream-coloured needles (100 mg.), m. p. and m. p. on admixture with an authentic sample, 215—217°.

(*c*) Methylation of 3'-hydroxy-5 : 7-dimethoxyflavone with methyl sulphate and aqueous sodium hydroxide furnished the *trimethyl ether* in colourless felted needles, m. p. 148° (from aqueous alcohol) [Found : C, 69·3; H, 5·2; OMe, 29·6. $C_{15}H_7O_2(OMe)_3$ requires C, 69·2; H, 5·2; OMe, 29·8%]. This compound (50 mg.) was refluxed in hydrobromic acid (48% w/w; 2 ml.) for 30 min. and the product isolated by dilution with water. Digestion of this with 2N-aqueous sodium hydroxide at the centrifuge yielded a liquor which was acidified, and the resulting precipitate recrystallised rom alcohol. 5 : 3'-Dihydroxy-7-methoxyflavone was obtained in yellow prisms (12 mg.), m. p. and mixed m. p. 236—238°.

Selective Methylation with Sodium Hydrogen Carbonate in Acetone.—(*a*) 7 : 3'-Dihydroxyflavone (500 mg.), sodium hydrogen carbonate (10 g.), methyl sulphate (275 mg., 1·1 mols.), and acetone (100 ml.) were refluxed with stirring in a slow stream of carbon dioxide. After 24 hr. the mixture was filtered, the solids were washed with boiling acetone, and the combined filtrate and washings reduced to low bulk and diluted with water. A chromatogram on a portion of the resulting precipitate showed it to consist largely of 7-monomethyl ether with small amounts of dimethyl ether and starting material. This material was triturated with 10% aqueous sodium hydroxide, filtered to remove dimethyl ether, and acidified. The resulting solid (350 mg.) had m. p. 242—245°. Recrystallisation from alcohol furnished 3'-hydroxy-7-methoxyflavone in cream-coloured needles, m. p. and mixed m. p. 249—250°.

(*b*) 3 : 7-Dihydroxyflavone (500 mg.) was methylated as above. Thrice recrystallised from alcohol, the crude reaction product yielded 3-hydroxy-7-methoxyflavone in cream-coloured needles (210 mg.), m. p. and mixed m. p. 179·5—180·5°.

(*c*) 3 : 7 : 3'-Trihydroxyflavone (100 mg.) was methylated by the same procedure. Acidification of the alkali-soluble portion of the product yielded a solid which on repeated recrystallisation from alcohol furnished 3 : 3'-dihydroxy-7-methoxyflavone in pale yellow needles (40 mg.), m. p. and mixed m. p. 214—216°.

(*d*) The product which was obtained when galangin (150 mg.) was methylated in the same way had m. p. 182—190°. Repeated recrystallisation from alcohol furnished 3 : 5-dihydroxy-7-methoxyflavone (55 mg.), m. p. and mixed m. p. 195—196°.

(*e*) 7 : 4'-Dihydroxyflavone (500 mg.) was methylated during 48 hr. by the same procedure. The alkali-insoluble portion of the product (80 mg.; m. p. 143—144°) proved to be 7 : 4'-dimethoxyflavone. The alkali-soluble portion was separated by repeated chromatography on Whatman No. 1 paper into unchanged starting material (55 mg.) and a mixture of the two monomethyl ethers. The latter solid (360 mg.) had m. p. 255—259°. Visual comparison of the colour of its fluorescence in ultra-violet light with the fluorescence colours of known mixtures of 7- and 4'-monomethyl ether suggested that its composition was 7—8 parts of 7- to 2—3 parts

of 4′-monomethyl ether. Repeated recrystallisation from alcohol furnished pure 4′-hydroxy-7-methoxyflavone in slender pale yellow prisms, m. p. and mixed m. p. 267°.

Selective Methylation in Sodium Carbonate Solution.—(*a*) Methyl sulphate (3·5 ml.) was added to a solution of 7 : 3′-dihydroxyflavone (150 mg.) in alcohol (20 ml.) and N-aqueous sodium carbonate (35 ml.). After 20 min. at 20°, the solution was diluted with water, filtered to remove a trace of dimethyl ether, and acidified. The solid was collected and a further quantity obtained by evaporation (*in vacuo*) of the mother-liquor. The combined solids (135 mg.) had m. p. 274—278°. A chromatogram run on Whatman's No. 1 paper with benzene–pyridine–water showed this material to consist of 7-hydroxy-3′-methoxyflavone contaminated with a trace of dihydroxyflavone and a blue fluorescent compound which was not identified. After 3 recrystallisations from alcohol, the pure monomethyl ether was obtained in cream-coloured needles, m. p. and mixed m. p. with an authentic sample, 282—284°.

(*b*) The product (120 mg.) which was obtained when 3 : 7-dihydroxyflavone (150 mg.) was methylated during 15 min. and the mixture was worked up as described above had m. p. 225—230°. Recrystallised from ethyl acetate and then from aqueous methanol, it furnished 7-hydroxy-3-methoxyflavone in colourless felted needles, m. p. and mixed m. p. 234—236°.

(*c*) Galangin (20 mg.) was methylated during 15 min. by the same reactants in proportionate quantities. Worked up as above, it yielded a solid (15 mg.), m. p. 285—290°, which on recrystallisation from ethyl acetate–light petroleum and then from alcohol gave 5 : 7-dihydroxy-3-methoxyflavone in pale yellow prisms, m. p. and mixed m. p. 297—299°.

(*d*) 3 : 4′-Dihydroxyflavone (150 mg.) was methylated by the same procedure. The alkali-soluble portion (80 mg.) of the product was repeatedly recrystallised from alcohol, furnishing 4′-hydroxy-3-methoxyflavone in cream-coloured prisms (15 mg.), m. p. and m. p. on admixture with an authentic specimen 230—233°.

Methylation of 7 : 4′-Dihydroxyflavone with Potassium Carbonate and Methyl Sulphate in Acetone.—(*a*) This compound (500 mg.), methyl sulphate (275 mg., 1·1 mols.), acetone (100 ml.), and potassium carbonate (10 g.) were refluxed, with stirring for 24 hr. Worked up by the standard method, the product yielded an alkali-insoluble residue (220 mg.), m. p. 140—144°, raised to 143—144° on recrystallisation from methanol, which consisted of 7 : 4′-dimethoxyflavone. The alkali-soluble portion (270 mg.) had m. p. approx. 310° (decomp.). A paper chromatogram showed this material to be 7 : 4′-dihydroxyflavone, contaminated with a little monomethyl ether.

7-Hydroxy-3′-methoxyflavone.—Condensation of 4-benzyloxy-2-hydroxyacetophenone (4 g.) with *m*-methoxybenzaldehyde (6 g.) and aqueous sodium hydroxide (6 g. in water, 12 ml.) in alcohol (20 ml.) during 24 hr. yielded *4-benzyloxy-2-hydroxyphenyl 3-methoxystyryl ketone* (6·5 g.), yellow prisms (from alcohol), m. p. 141—142°, giving a reddish-brown ferric colour in alcohol (Found : C, 76·6; H, 5·4. $C_{23}H_{20}O_4$ requires C, 76·7; H, 5·6%). It (5 g.) was then heated with selenium dioxide (resublimed; 7 g.) in amyl alcohol (80 ml.) under reflux for 18 hr. and filtered. The combined filtrate and washings were steam-distilled, leaving a residue which on recrystallisation from alcohol yielded *7-benzyloxy-3′-methoxyflavone* in colourless needles (3·5 g.), m. p. 161·5—162·5° (Found : C, 77·0; H, 5·0. $C_{23}H_{18}O_4$ requires C, 77·1; H, 5·1%). Debenzylation of this material (3 g.) was effected by acetic acid (30 ml.) and concentrated hydrochloric acid (25 ml.) on the water-bath for 1 hr. After steam-distillation and recrystallisation of the residue from alcohol, *7-hydroxy-3′-methoxyflavone* was obtained in cream-coloured needles (1·9 g.), m. p. 282—285° (Found : C, 17·6; H, 4·5; OMe, 11·9. $C_{15}H_9O_3$·OMe requires C, 71·6; H, 4·5; OMe, 11·6%). Its *acetate* separated from methanol in colourless prisms, m. p. 144—145° (Found : C, 69·6; H, 4·3. $C_{18}H_{14}O_5$ requires C, 69·7; H, 4·5%).

4′-Hydroxy-3-methoxyflavone.—*4-Benzyloxystyryl 2-hydroxyphenyl ketone* was prepared by condensing *o*-hydroxyacetophenone (5 g.) with *p*-benzyloxybenzaldehyde (9 g.) and sodium hydroxide (12 g. in the minimum of water) in alcohol (40 ml.). It separated from alcohol in pale yellow, lustrous plates (8 g.) m.p . 112—113°, giving a brown alcoholic ferric colour (Found : C, 80·1; H, 5·6. $C_{22}H_{18}O_3$ requires C, 80·0; H, 5·5%). To a solution of this compound (1 g.) in hot alcohol (15 ml.), N-alcoholic potassium hydroxide (7 ml.) was added, followed immediately by hydrogen peroxide (100-vol.; 2 ml.). After 15 min. the mixture was diluted with water and acidified. The resulting solid was recrystallised from alcohol, yielding *4′-benzyloxy-3-hydroxyflavone* in pale yellow needles (450 mg.), m. p. 176·5—177·5°, giving a violet ferric colour in alcohol (Found : C, 76·7; H, 4·9. $C_{22}H_{16}O_4$ requires C, 76·7; H, 4·7%). Its *acetate* separated from alchol in colourless plates, m. p. 145—146° (Found : C, 74·6; H, 4·7. $C_{24}H_{18}O_5$ requires C, 74·6; H, 4·7%). Methylation of the benzyl ether (2 g.) with an excess of methyl sulphate and potassium carbonate in boiling acetone was complete in 12 hr. and yielded *4′-benzyloxy-3-*

methoxyflavone. Recrystallised from alcohol, this formed colourless plates (1·5 g.), m. p. 112—113°, which gave no ferric colour (Found: C, 76·9; H, 5·0%). This material (2·8 g.) was debenzylated by acetic acid (25 ml.) and concentrated hydrochloric acid (15 ml.) on a water-bath for 1 hr. *4'-Hydroxy-3-methoxyflavone* was obtained in cream-coloured prisms (1·7 g.), m. p. 232—233°, from alcohol (Found: C, 71·8; H, 4·6; OMe, 11·7%). Its *acetate* formed colourless needles (from methanol), m. p. 138—139° (Found: C, 69·9; H, 4·4%).

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