Anal. Calcd. for $C_{17}H_{16}O_4$: C, 71.8; H, 5.68; 1 CH_3CO–, 15.2. Found: C, 71.6; H, 5.67; CH_3CO–, 14.7.

4-O-Benzylresacetophenone.—The above product (1.7 g.) was hydrolyzed by adding 10% aqueous sodium hydroxide (20 ml.) to its solution in boiling methanol (10.0 ml.). After 5 minutes the solution was cooled, diluted with water (20 ml.) and acidified with hydrochloric acid. The solid precipitate was collected and crystallized from methanol. 4-O-Benzylresacetophenone crystallized in colorless plates, m.p. 104–104.5° (1.1 g.). It gave an intense red-brown color with methanolic ferric chloride.

Anal. Calcd. for $C_{15}H_{14}O_3$: C, 74.3; H, 5.83. Found: C, 74.3; H, 5.81.

A mixture of the 4-O-benzylresacetophenone (1.0 g.), methyl iodide (10.0 ml.), potassium carbonate (5.0 g.) and acetone (40 ml.) was refluxed for 17 hours. The filtered acetone solution was evaporated and the residue was crystallized from hexane. 2-O-Methyl-4-O-benzylresacetophenone separated in colorless plates, m.p. 72° (0.85 g.).

Anal. Calcd. for $C_{16}H_{16}O_3$: C, 75.0; H, 6.30. Found: C, 75.3; H, 6.62.

A solution of the methyl ether (1.8 g.) in glacial acetic acid (20 ml.) and concentrated hydrochloric acid (10 ml.) was heated on a steam-bath for 30 minutes. Excess of water was added and the product was extracted with ether. The ether solution was washed with water and dilute sodium bicarbonate solution, dried (Na_2SO_4) and evaporated. The oil crystallized from benzene–hexane and the product was recrystallized from water. 2-O-Methylresacetophenone thereby separated in colorless needles, m.p. 138° (lit.[18] m.p. 138°) which dissolved in dilute alkali but did not give a ferric chloride reaction.

5-Acetylquinacetophenone.—Quinacetophenone was acetylated by the method described for the acetylation of resacetophenone. The acetate, recrystallized from methanol, was obtained in slightly yellow blades, m.p. 91° (5-acetylquinacetophenone, lit.[19] m.p. 91°), which gave a strong red-brown ferric reaction.

(19) Reference 14, p. 292.

Anal. Calcd. for $C_{10}H_{10}O_4$: C, 61.8; H, 5.56. Found: C, 62.2; H, 5.41.

2-O-Benzyl-5-acetylquinacetophenone.—5-Acetylquinacetophenone (1.0 g.) was benzylated by refluxing with benzyl chloride (1.0 ml.), potassium iodide (0.1 g.), anhydrous potassium carbonate (2.0 g.) and dry acetone (30 ml.) for 3 hours. The oil which remained on evaporation of the filtered acetone solution was dissolved in hot hexane (70 ml.) On concentration and cooling the hexane solution deposited colorless crystals and a little oily material. The hexane mother liquor was decanted and the oil was dissolved by washing the crystals rapidly with cold methanol. The crystalline material then was recrystallized from methanol. Colorless glistening blades were thus obtained, m.p. 88° (0.7 g.).

Anal. Calcd. for $C_{17}H_{16}O_4$; C, 71.8; H, 5.68; 1 CH_3CO–, 15.2. Found: C, 71.9; H, 5.67; CH_3CO–, 15.0.

2-O-Benzylquinacetophenone.—The above product (0.68 g.) was hydrolyzed in warm 5% aqueous methanolic sodium hydroxide. On dilution with water and acidification, a crystalline product separated. This was recrystallized from methanol containing a little water. 2-O-Benzylquinacetophenone was obtained as slightly yellow needles, m.p. 117° (0.4 g.), which dissolved in dilute aqueous alkali to give a yellow solution. It did not give a color with alcoholic ferric chloride.

Anal. Calcd. for $C_{15}H_{14}O_3$: C, 74.3; H, 5.83. Found: C, 74.4; H, 5.83.

Ultraviolet Spectra.—Ultraviolet spectra were determined in absolute ethanol at room temperature on a Cary recording spectrophotometer.

Acknowledgments.—The authors are indebted to L. M. White for performing the elementary analyses.

PASADENA, CALIF.

[CONTRIBUTION FROM THE FRUIT AND VEGETABLE CHEMISTRY LABORATORY, WESTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION, AGRICULTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

Plant Polyphenols. V. Selective Alkylation of the 7-Hydroxyl Group in Polyhydroxyflavones

BY LEONARD JURD[1]

RECEIVED MARCH 14, 1958

Quercetin pentaacetate reacts with methyl iodide or benzyl chloride in dry acetone in the presence of potassium carbonate to give high yields of rhamnetin tetraacetate and 7-O-benzylquercetin tetraacetate, respectively. Further benzylation of 7-O-benzylquercetin tetraacetate in methyl ethyl ketone gives 3,4′,5,7-tetra-O-benzylquercetin monoacetate which yields isorhamnetin when successively deacetylated, methylated and debenzylated. 3,4′,7-Tri-O-benzylquercetin 5-monoacetate and 4′,7-di-O-benzylquercetin triacetate were isolated as by-products in these reactions. From these data it follows that acetyl groups attached to the flavone nucleus may be successively replaced by alkyl groups in the order 7 > 4′ > 3 > 5 > 3′. The constitutions of these new partial benzyl ethers of quercetin were established by recently reported spectrophotometric procedures.

The 7-alkyl ethers of certain flavonols, *e.g.*, rhamnetin, recently have achieved some importance as potential anti-oxidants for unsaturated fats.[2–4] However, few methods are at present known for the selective partial alkylation of polyhydroxyflavones in the laboratory, although partially alkylated flavones, particularly 7-methoxyflavones, occur frequently in plants.[5] The direct alkylation of phenolic flavones with excess of the usual reagents results in complete O-alkylation, alkylation of all phenolic groups except the chelated 5-hydroxyl, or nuclear alkylation.[6–12] *o*-Dihydroxyl groups may be protected during methylation by

(1) Financial support for this work was provided by the Diamond Walnut Growers, Inc.

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(4) W. H. Heimann and F. Reiff, *Fette u. Seifen*, **55**, 451 (1953).

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TABLE I

ULTRAVIOLET SPECTRA OF QUERCETIN DERIVATIVES

Compound	λ_{max}, mμ EtOH	NaOAc	$\Delta\lambda^1$	H_3BO_3–NaOAc	$\Delta\lambda^2$	0.002 *M* NaOEt	$AlCl_3$[a]	$\Delta\lambda^3$
Quercetin	371	388		388	17	325	431	60
		334						
	257	278	21	260		242	268	
Rhamnetin (authentic and from quercetin pentaacetate)	372	383		388	16	361		
	258	258	0	261		296		
7-O-Benzylquercetin	372	379		386	14	368		
	258	259	1	261		294		
3,3′,4′,5-Tetra-O-methylquercetin	341	349				363		
	249	272	23			275		
Quercetin pentaacetate	300						300	0
	252						252	
Rhamnetin tetraacetate (authentic and from quercetin pentaacetate)	306						306	0
	254						254	
7-O-Benzylquercetin tetraacetate	306						306	0
	254						254	
3,3′,4′,5-Tetra-O-methylquercetin monoacetate	342							
	248							
Isorhamnetin tetraacetate	311							
	241							
3,4′,7-Tri-O-benzylquercetin diacetate	330							
	259							
3,4′,7-Tri-O-benzylquercetin 5-monoacetate	348						343	−5
	278						281	
Quercetin 3,3′,4′,7-tetraacetate	335						385	50
	268						285	
Rhamnetin 3,3′,4′-triacetate	340[b]						392	52
	304						329	
	268						282	
4′,7-Di-O-benzylquercetin triacetate	314							
	255							
	235[b]							
3,4′,5,7-Tetra-O-benzylquercetin monoacetate	332							
	267							

$\Delta\lambda' = \lambda_{max}(NaOAc) - \lambda_{max}(EtOH)$

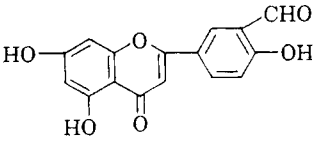
$\Delta\lambda^2 = \lambda_{max}(H_3BO_3/NaOAc) - \lambda_{max}(EtOH)$

$\Delta\lambda^3 = \lambda_{max}(AlCl_3) - \lambda_{max}(EtOH)$

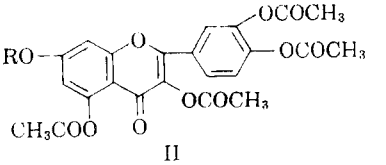
[a] 4 drops of 10% aqueous $AlCl_3$ added to cell. [b] Inflection.

chelation with borax,[13] while simple flavones which contain only the acidic 7-hydroxyl group and less acidic 3- or 3′-hydroxyl groups may be preferentially methylated in the 7-position by reaction with one equivalent of dimethyl sulfate.[14] The methylation rates of 7- and 4′-hydroxyl groups and 3- and 4′-hydroxyl groups, however, do not differ sufficiently for the satisfactory partial methylation of flavones containing hydroxyl groups in those positions. In order to achieve partial methylation in the 7-position only, Simpson[15] recently attempted to synthesize and methylate formylflavones of the type I in which the 4′-hydroxyl is protected by chelation. The yields of the intermediate formylflavones, however, were too low (5–10%) to be useful.

In this investigation it has been found that the direct alkylation of quercetin with limited quantities of alkyl halides gives mixtures of unchanged quercetin and highly O-alkylated products. When quercetin pentaacetate (II, R = CH_3CO–) is treated with excess of methyl iodide and potassium carbonate in *dry* acetone, however, one acetyl group only is replaced and rhamnetin tetraacetate (II, R = –Me) is obtained in 80% yields. Alkaline hydrolysis of the tetraacetate gives rhamnetin. The identity of this product as rhamnetin was established analytically, by direct comparison by



I



II

mixed melting point of its tetraacetate and authentic rhamnetin tetraacetate, and spectrophotometrically. The spectral data for the flavonol and authentic rhamnetin are given in Table I. The low wave length band is not shifted in the presence of sodium acetate showing that the hydroxyl at position 7 is methylated.[16]

Quercetin pentaacetate reacts similarly with excess of benzyl chloride in *dry* acetone to give a

(13) A. C. Jain, K. S. Pankajamani and T. R. Seshadri, *J. Sci. Ind. Research (India)*, **12B**, 127 (1953).

(14) T. H. Simpson and J. L. Beton, *J. Chem. Soc.*, 4065 (1954).

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monobenzylquercetin tetraacetate. On alkaline hydrolysis this forms a monobenzylquercetin, m.p. 245–246°. Direct comparison of the spectra (Table I) and other properties of this monobenzylquercetin with those of 7-O-benzylquercetin[17] proved them to be identical. This was confirmed by methylating the monobenzylquercetin to give a monobenzyltetramethylquercetin. Debenzylation of this gave 3,3′,4′,5-tetra-O-methylquercetin, identical with the product[9,17] obtained from 7-O-benzylquercetin.

Acetyl groups located elsewhere on the flavone nucleus may be replaced by using the higher boiling methyl ethyl ketone as the solvent in the reaction. Thus 7-O-benzylquercetin tetraacetate reacts with benzyl chloride in dry methyl ethyl ketone to give an O-tetrabenzylquercetin monoacetate, m.p. 176°. Alkaline hydrolysis of this gives an O-tetrabenzylquercetin, m.p. 166°. The positions of the absorption bands (Fig. 1) of this ether are not

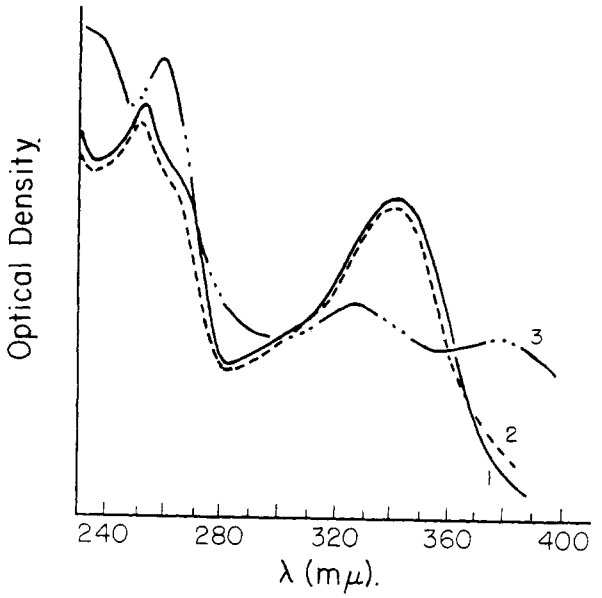
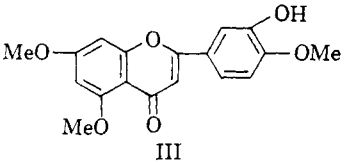
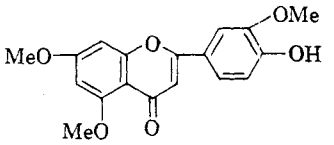


Fig. 1.—Ultraviolet spectrum of 3,4′,5,7-tetra-O-benzylquercetin in: 1, absolute ethanol; 2, ethanolic sodium acetate; 3, 0.002 *M* sodium ethylate.

altered on the addition of aluminum chloride,[18,19] sodium acetate[16] or boric acid–sodium acetate.[20] The hydroxyl groups at positions 3, 5 and 7 are therefore benzylated and the fourth benzyl group is located at either the 3′- or the 4′-position. The spectral data strongly favor the location of this benzyl group at the 4′-position for two reasons, *viz.*, (1) ionization of either a 3′- or a 4′-hydroxyl produces a bathochromic shift of the long wave length band. An hydroxyl group at the 3′-position is not sufficiently acidic to be ionized by the weakly basic sodium acetate. An hydroxyl group at the 4′-position, on the other hand, is strongly acidic and consequently is usually partially ionized by sodium acetate. The long wave length band of the tetra-O-benzylquercetin is unaffected by sodium acetate suggesting, therefore, the absence of a free 4′-hydroxyl. (2) Ionization of the free hydroxyl group of the tetra-O-benzylquercetin by sodium ethylate produces a 34 mμ bathochromic shift of the long wave length band. The small magnitude of this shift indicates that it results from the ionization of an unconjugated 3′-hydroxyl rather than a highly conjugated 4′-hydroxyl group. In agreement with this Nordström and Swain[21] have shown that the λ_{max} of the closely related compound 3′-hydroxy-4′,5,7-trimethoxyflavone (III) (λ_{max} 334 mμ) shifts only 46 mμ in so-



III



IV

dium ethylate whereas the isomeric compound, 4′-hydroxy-3′,5,7-trimethoxyflavone (IV) (λ_{max} 335 mμ) shifts 65 mμ. The spectrophotometric evidence, therefore, indicates the structure 3,4′,5,7,-tetra-O-benzylquercetin (V, R = $C_6H_5CH_2$–, R_1 = H) for the benzylation product. Methylation of the tetra-O-benzylquercetin and subsequent debenzylation gives a monomethylquercetin, m.p. 305°, which forms a tetraacetate, m.p. 205°. These values agree closely with those reported[22] for isorhamnetin (V, R = H, R_1 = Me) (m.p. 305°) and its tetraacetate (m.p. 205–207°). An authentic sample of isorhamnetin was not available for direct comparison. However, the spectra (Fig. 2) of the monomethylquercetin clearly confirm its identity with isorhamnetin. Thus fused sodium acetate produces a bathochromic shift (20

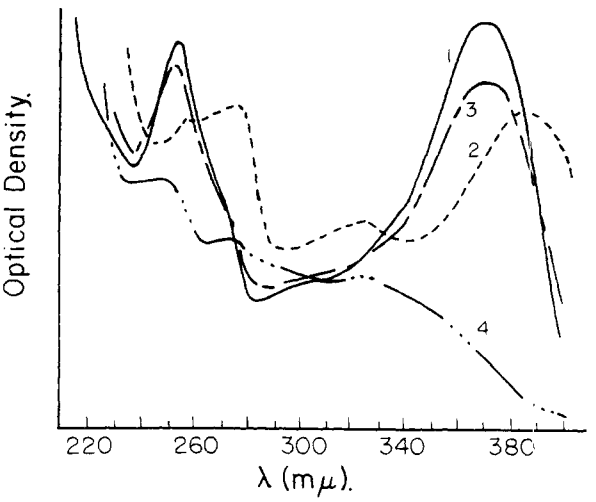


Fig. 2.—Ultraviolet spectrum of isorhamnetin in: 1, absolute ethanol; 2, ethanolic sodium acetate; 3, ethanolic boric acid–sodium acetate; 4, 0.002 *M* sodium ethylate.

(17) Part IV of this series, L. Jurd, THIS JOURNAL, **80**, 5527 (1958).
(18) T. Swain, *Chemistry & Industry*, 1480 (1954).
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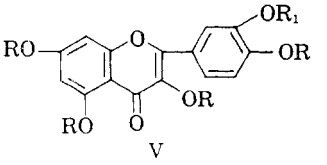
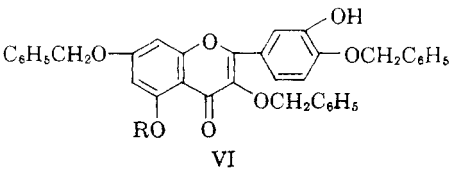
(20) L. Jurd, *Arch. Biochem. and Biophys.*, **63**, 376 (1956).
(21) C. G. Nordström and T. Swain, *J. Chem. Soc.*, 2764 (1953).
(22) "Dictionary of Organic Compounds," eds. I. Heilbron and H. M. Bunbury, Vol. 3, Oxford University Press, New York, 1953, p. 125.

mμ) of the low wave length band, showing that the 7-hydroxyl is free. The long wave length band disappears in sodium ethylate indicating that the hydroxyls at the 3- and 4′-positions are free. Boric acid–sodium acetate does not shift the long wave length band showing that monomethylquercetin does not have a free *o*-dihydroxyl group. Since the hydroxyl at 4′ is free, the methoxyl group must therefore be located at 3′. The identification of the monomethylquercetin as isorhamnetin confirms the structure V (R = $C_6H_5CH_2$–, R_1 = H) assigned to the tetra-O-benzylquercetin.

During this investigation a tri-O-benzyl- and a di-O-benzylquercetin also were isolated. The structures assigned to these compounds establish the order of replacement of each of the acetoxy groups on the flavone nucleus. The tribenzyl ether was isolated in one experiment on the benzylation of quercetin pentaacetate in acetone which had not been dried over potassium carbonate. Analysis of the product showed that it was a tri-O-benzylquercetin monoacetate. Acetylation and methylation of this monoacetate gave a tri-O-benzylquercetin diacetate and a mono-O-methyltri-O-benzylquercetin monoacetate, respectively. Hydrolysis of the monoacetate produced the tri-O-benzylquercetin, m.p. 140°. Analysis of the spectra (Fig. 3) of this compound indicates its constitution to be 3,4′,7-tri-O-benzylquercetin (VI, R = H). The low λ_{max} (353 mμ) in alcohol and the absence of shifts with sodium acetate and boric acid–sodium acetate show that the hydroxyls at 3,7 and 4′ are benzylated. The aluminum chloride spectrum is typical for those flavones whose only chelatogenic grouping is a free 5-hydroxyl[19] while the small magnitude of the bathochromic shift of the long wave length band in sodium ethylate indicates ionization of a free 3′-hydroxyl group (ref. 21). Aluminum chloride does not produce a bathochromic shift of the long wave length band of the monoacetate (Table I). This indicates that the compound is the 3,4′,7-tri-O-benzylquercetin 5-monoacetate (VI, R = CH_3CO–). In this connection it should be noted that aluminum chloride has no effect on the spectra of quercetin penta-



V



VI

acetate or rhamnetin tetraacetate (Table I) in which all hydroxyls are substituted, or on 3,4′,5,7-tetra-O-benzylquercetin in which the 3′-hydroxyl group is free. With quercetin 3,3′,4′,7-tetraacetate and rhamnetin 3,3′,4′-triacetate, each of which contains a free 5-hydroxyl group, aluminum chloride produces a bathochromic shift (about 50 mμ) of the long wave length band (Table I).

The benzylation of 7-O-benzylquercetin tetraacetate in methyl ethyl ketone to give 3,4′,5,7-tetra-O-benzylquercetin monoacetate has been repeated a number of times. However in one experiment, conducted under apparently identical conditions, the product was a dibenzylquercetin triacetate, m.p. 152–153°. The di-O-benzylquercetin obtained on hydrolysis of the triacetate was stable in sodium ethylate, showing that either the 3- or 4′-hydroxyl was benzylated. Since boric acid–sodium acetate did not give a bathochromic shift (Fig. 4) it is the 4′-hydroxyl which is benzylated. This conclusion is confirmed by the large bathochromic shift characteristic of a free 3-hydroxyl group in the presence of aluminum chloride.

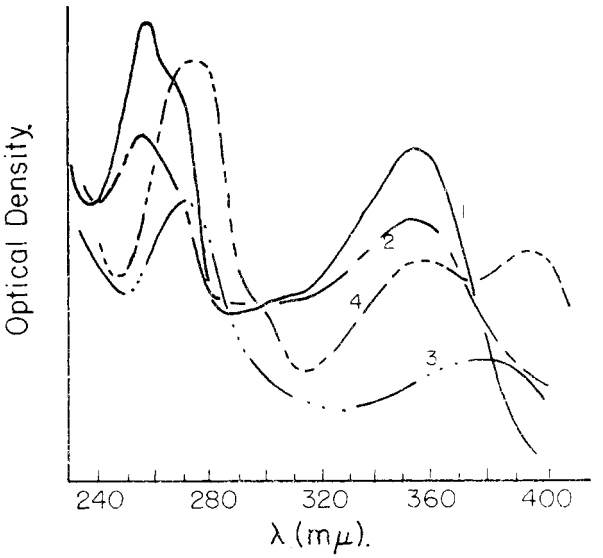


Fig. 3.—Ultraviolet spectrum of 3,4′,7-tri-O-benzylquercetin in: 1, absolute ethanol; 2, ethanolic boric acid–sodium acetate; 3, 0.002 *M* sodium ethylate; 4, ethanolic aluminum chloride.

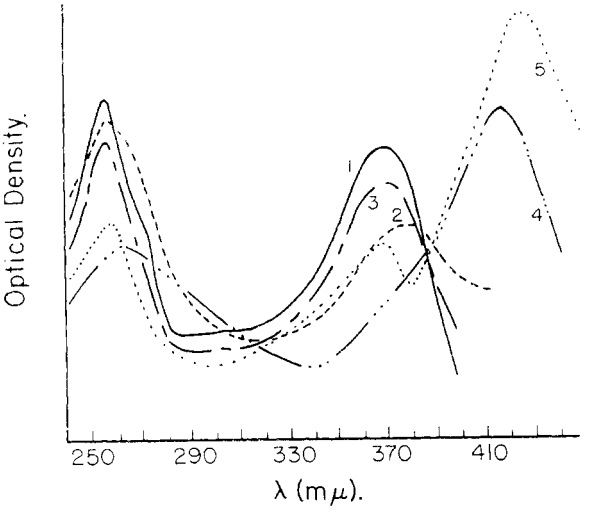
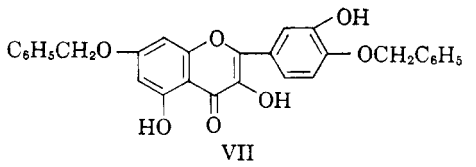


Fig. 4.—Ultraviolet spectrum of 4′,7-di-O-benzylquercetin in: 1, absolute ethanol; 2, ethanolic sodium acetate; 3, ethanolic boric acid–sodium acetate; 4, 0.002 *M* sodium ethylate; 5, ethanolic aluminum chloride.

The dibenzyl compound is therefore 4',7-di-O-benzylquercetin (VII). Thus the partial benzylation of quercetin pentaacetate produces the acetates of 7-O-benzyl-, 4',7-di-O-benzyl-, 3,4',7-tri-O-benzyl- and 3,4',5,7-tetra-O-benzylquercetin. The order of replacement of acetoxy groups attached to the flavone nucleus, therefore, lies in the order $7 > 4' > 3 > 5 > 3'$. It is apparent that the di- and tribenzyl ethers obtained as side-products in this work will be useful for the synthesis of partial methyl ethers of quercetin. Further work, therefore, is being undertaken to define more closely the conditions necessary for their large scale preparation.



VII

Experimental

Rhamnetin Tetraacetate (II, R = Me).—A mixture of quercetin pentaacetate (5.0 g.), methyl iodide (10.0 ml.), anhydrous potassium carbonate (13 g.) and anhydrous acetone (75 ml.) was heated under reflux for 20 hours. The undissolved potassium salts were filtered and the filtrate was evaporated to a gum. This was dissolved in warm benzene (50 ml.), the solution was filtered and diluted with hexane. The benzene–hexane solution was concentrated until crystallization began. After cooling, the crystalline product (4.2 g., 89%) was collected and recrystallized from acetone–methanol. Colorless needles, m.p. 189–190°, were thus obtained (3.2 g., 68%).

Anal. Calcd. for $C_{24}H_{20}O_{11}$: C, 59.5; H, 4.16; 1 MeO–, 6.44; 4 CH_3CO–, 35.5. Found: C, 60.0; H, 4.39; MeO–, 6.94; CH_3CO–, 35.1.

An authentic sample of rhamnetin,[23] purified by recrystallization from acetone–methanol, was acetylated to give rhamnetin tetraacetate, m.p. 190°, undepressed on admixture with the above product.

Rhamnetin.—Aquous sodium hydroxide (3.5 ml., 10%) was added to a suspension of rhamnetin tetraacetate (3.1 g.) in warm methanol (15.0 ml.). After 3 minutes water (10 ml.) was added and heating was continued for 5 minutes. The mixture was acidified with concentrated hydrochloric acid (2.5 ml.) and digested on the steam-bath for 40 minutes. The crystalline product which separated was collected and recrystallized from acetone–methanol. Rhamnetin was thus obtained as yellow needles, m.p. 292–294° (lit. m.p. 294–296°) (1.6 g., 79%).

Anal. Calcd. for $C_{16}H_{12}O_7$: C, 60.8; H, 3.83; 1 MeO–, 9.87. Found: C, 60.9; H, 4.01; MeO–, 9.98.

On Whatman No. 1 paper both the product and authentic rhamnetin had R_f 0.41 in 50% aqueous acetic acid. Quercetin had R_f 0.31. Reacetylation of the product with acetic anhydride and sodium acetate gave rhamnetin tetraacetate, m.p. 190°.

7-O-Benzylquercetin Tetraacetate (II, R = $C_6H_5CH_2$–).—A mixture of quercetin pentaacetate (10.0 g.), benzyl chloride (10.0 ml.), potassium iodide (1.0 g.), anhydrous potassium carbonate (25 g.) and anhydrous acetone (250 ml.) was heated under reflux for 21 hours. The acetone solution was filtered from undissolved potassium salts and concentrated to an oil. This was washed with hexane (3 × 50 ml.) by decantation. The gummy residue was dissolved in warm benzene (100 ml.), the solution was filtered and the filtrate was treated slowly at boiling point with hexane (150 ml.) until crystallization began. The mixture was cooled and the colorless crystalline product (10.3 g.) was collected. Crystallized from beuzene–hexane 7-O-benzylquercetin tetraacetate separated in colorless needles, m.p. 113–115° (7.5 g., 69%). It crystallized from methanol–acetone in brittle, slightly yellow needles, m.p. 163°, undepressed on admixture with the 7-O-benzylquercetin tetraacetate obtained by the benzylation of quercetin 3,3',4',7-tetraacetate.

Anal. Calcd. for $C_{30}H_{24}O_{11}$: C, 64.2; H, 4.32; 4 CH_3CO–, 30.7. Found: C, 64.4; H, 4.4.0; CH_3CO–, 30.3.

7-O-Benzylquercetin.—10% Aqueous sodium hydroxide (36 ml.) was added to a suspension of 7-O-benzylquercetin tetraacetate (10.0 g.) in hot methanol (100 ml.). The solution was heated for 2 minutes, water (100 ml.) was added and heating continued for 3 minutes when a crystalline sodium salt separated. Concentrated hydrochloric acid (20.0 ml.) then was added slowly whereupon a yellow crystalline solid precipitated. Water (300 ml.) was added and the solid was collected. Recrystallized from methanol–acetone 7-O-benzylquercetin was obtained in yellow needles, m.p. and mixed m.p. 245–247° (5.6 g., 80%).

A solution of the 7-O-benzylquercetin (50 mg.) in pyridine (0.5 ml.) was treated with benzoyl chloride (0.2 ml.). The mixture was heated on the steam-bath for 10 minutes, allowed to stand for 3 hours and added to excess of water. The solid benzoate was collected and recrystallized from acetone–methanol. 7-O-Benzylquercetin tetrabenzoate thereby separated in colorless needles, m.p. 236°.

Anal. Calcd. for $C_{50}H_{32}O_{11}$: C, 74.2; H, 3.91. Found: C, 74.3; H, 4.07.

Debenzylation of 7-O-Benzylquercetin.—A solution of 7-O-benzylquercetin (0.20 g.) in tetrahydrofuran (20.0 ml.) was hydrogenated at atmospheric pressure in the presence of a 30% palladium–charcoal catalyst (0.1 g.). The catalyst was removed and the filtrate was evaporated. The yellow crystalline residue was recrystallized from methanol–acetone. Quercetin, m.p. and mixed m.p. 310°, was obtained.

Anal. Calcd. for $C_{15}H_{10}O_7$: C, 59.6; H, 3.57. Found: C, 59.2; H, 3.48.

The acetate of this product separated from acetone–methanol in colorless needles, m.p. 201–202°, undepressed on admixture with quercetin pentaacetate.

Anal. Calcd. for $C_{25}H_{20}O_{12}$: C, 58.5; H, 3.94; 5 CH_3CO–, 42.0. Found: C, 58.7; H, 4.10; CH_3CO–, 41.8.

3,3',4',5-Tetra-O-methylquercetin.—7-O-Benzylquercetin (2.9 g.) was methylated with methyl iodide, potassium carbonate and acetone as previously described.[17] 7-O-Benzyl-3,3',4',5-tetra-O-methylquercetin separated from benzene–hexane in colorless needles, m.p. and mixed m.p. 169–171° (2.3 g.).

The above product (0.5 g.), dissolved in a mixture of glacial acetic acid (22.0 ml.) and concentrated hydrochloric acid (11.0 ml.), was debenzylated as previously described. 3,3',4',5-Tetra-O-methylquercetin separated from glacial acetic acid–ethyl acetate in slightly yellow needles, m.p. and mixed m.p. 283–284° (0.17 g.). **The acetate** of this compound separated from acetone–methanol in colorless needles, m.p. and mixed m.p. 172°.

Anal. Calcd. for $C_{21}H_{20}O_8$: C, 63.0; H, 5.04; 4 MeO–, 31.0; 1 CH_3CO–, 10.7. Found: C, 63.6; H, 5.12; MeO–, 30.2; CH_3CO–, 10.6.

3,4',5,7-Tetra-O-benzylquercetin Monoacetate (V, R = $C_6H_5CH_2$–, R_1 = CH_3CO–).—A mixture of 7-O-benzylquercetin tetraacetate (2.0 g.), benzyl chloride (4.0 ml.), potassium iodide (0.2 g.), anhydrous potassium carbonate (8.0 g.) and dry methyl ethyl ketone (40 ml.) was heated under reflux for 20 hours. The undissolved potassium salts were filtered and the filtrate was concentrated to an oil. This was dissolved in warm benzene and the solution treated with hexane until crystallization commenced. The product was recrystallized from acetone–methanol. Colorless needles, m.p. 176°, were thus obtained (0.8 g.).

Anal. Calcd. for $C_{45}H_{36}O_8$: C, 76.7; H, 5.15; 1 CH_3CO–, 6.13. Found: C, 76.8; H, 5.23; CH_3CO–, 6.04.

3,4',5,7-Tetra-O-benzylquercetin (V, R = $C_6H_5CH_2$–, R_1 = H.—The above product (0.8 g.) was dissolved in acetone (5.0 ml.). Methanol (10.0 ml.) and 10% aqueous sodium hydroxide (2.0 ml.) were added and the solution was heated on a steam-bath for 10 minutes. After dilution with water (30 ml.) and acidification the solid product was collected and recrystallized from acetone–methanol. It separated in slightly yellow needles, m.p. 166.5° (0.5 g.).

Anal. Calcd. for $C_{43}H_{34}O_7$: C, 77.9; H, 5.17. Found: C, 77.9; H, 5.23.

(23) Supplied by S. B. Penick and Co.

3'-O-Methyl-3,4',5,7-tetra-O-benzylquercetin (V, R = $C_6H_5CH_2$–, R_1 = Me).—The above product (0.5 g.) was methylated by refluxing with excess of methyl iodide and potassium carbonate in acetone in the usual way. The acetone solution was evaporated to a gum. This was treated with water and the colorless, undissolved solid was collected. Successively recrystallized from methanol and from benzene–hexane, 3'-O-methyl-3,4',5,7-tetra-O-benzylquercetin separated in colorless, hair-like needles, m.p. 126–127° (air-dried for several days).

Anal. Calcd. for $C_{44}H_{36}O_7$: C, 78.1; H, 5.37; 1 MeO–, 4.61. Found: C, 78.2; H, 5.34; MeO–, 4.48.

Isorhamnetin (V, R = H, R_1 = Me).—The above methyl ether (0.3 g.) was dissolved in a mixture of glacial acetic acid (10.0 ml.) and concentrated hydrochloric acid (5.0 ml.). The solution was heated on the steam-bath for 1.5 hours, cooled and diluted with water. The yellow solid (80 mg.) was collected and recrystallized from acetone–methanol. Isorhamnetin thus was obtained as yellow needles, m.p. 305–306°.

Anal. Calcd. for $C_{16}H_{12}O_7$: C, 60.7; H, 3.83; 1 MeO–, 9.87. Found: C, 60.7; H, 4.00; MeO–, 10.6.

The acetate of the product, prepared by heating it with acetic anhydride and sodium acetate, crystallized from acetone-methanol in colorless needles, m.p. 205°.

Anal. Calcd. for $C_{24}H_{20}O_{11}$: C, 59.5; H, 4.16; 1 MeO–, 6.44; 4 CH_3CO–, 35.5. Found: C, 59.9; H, 4.21; MeO–, 6.29; CH_3CO–, 35.2.

3,4',7-Tri-O-benzylquercetin.—Quercetin pentaacetate (2.0 g.), benzyl chloride (5.0 ml.) and anhydrous potassium carbonate (5.0 g.) were refluxed in acetone (undried) for 18 hours. The filtered acetone solution was concentrated to an oil which was dissolved in methanol (20 ml.). On standing a slightly yellow crystalline product (0.4 g.) separated. This was recrystallized from methanol–acetone and from benzene–hexane. 3,4',7-Tri-O-benzylquercetin 5-monoacetate (VI, R = CH_3CO–) separated in slightly yellow needles, m.p. 148–150°.

Anal. Calcd. for $C_{38}H_{30}O_8$: C, 74.2; H, 4.92. Found: C, 74.4; H, 5.14.

Acetylation of this monoacetate gave 3,4',7-tri-O-benzylquercetin diacetate. This crystallized from benzene–hexane in colorless needles, m.p. 161–163°.

Anal. Calcd. for $C_{40}H_{32}O_9$: C, 73.1; H, 4.92; 2 CH_3CO–, 14.0. Found: C, 73.0; H, 5.04; CH_3CO–, 12.2.

The monoacetate (0.1 g.) was methylated in dry acetone by refluxing with methyl iodide and potassium carbonate. The product was purified by recrystallization from benzene–hexane; 3'-O-methyl-3,4',7-tri-O-benzylquercetin monoacetate thus was obtained in colorless needles, m.p. 164–165°

Anal. Calcd. for $C_{39}H_{32}O_8$: C, 74.5; H, 5.14; 1 MeO–, 4.97. Found: C, 74.5; H, 5.38; MeO–, 5.11.

The monoacetate (50 mg.) was hydrolyzed in a mixture of warm methanol (3.0 ml.) and 10% aqueous sodium hydroxide (1.0 ml.). The solution was diluted with water and acidified. The solid product was recrystallized from acetone–methanol. Slightly yellow needles of 3,4',7-tri-O-benzylquercetin (VI, R = H) thus were obtained, m.p. 140°.

Anal. Calcd. for $C_{36}H_{28}O_7$: C, 75.5; H, 4.93. Found: C, 75.4; H, 4.93.

4',7-Di-O-benzylquercetin (VII).—7-O-Benzylquercetin tetraacetate (1.0 g.) was benzylated in dry methyl ethyl ketone as described previously. In this one experiment, however, the product was not 3,4',5,7-tetra-O-benzylquercetin monoacetate but a dibenzyl derivative. The product was crystallized successively from benzene–hexane and acetone–methanol. 4',7-Di-O-benzylquercetin triacetate thus was obtained as colorless needles, m.p. 152–153°.

Anal. Calcd. for $C_{35}H_{28}O_{10}$: C, 69.0; H, 4.64; 3 CH_3CO–, 21.2. Found: C, 69.0; H, 4.69; CH_3CO–, 20.3.

The triacetate was hydrolyzed in dilute methanolic sodium hydroxide, 4,7-di-O-benzylquercetin (VII) crystallized from acetone–methanol in brightly yellow needles, m.p. 179°, which gave an olive-green ferric reaction and did not reduce Tollens reagent immediately.

Anal. Calcd. for $C_{29}H_{22}O_7$: C, 72.2; H, 4.60. Found: C, 72.1; H, 4.60.

Ultraviolet Spectra.—All spectra were determined in absolute ethanol solutions on a Cary recording spectrophotometer.

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PASADENA, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

The Structure of Terreic Acid

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Terreic acid, an antibiotic metabolite of the mold *Aspergillus terreus*, has been converted by treatment with a mixture of acetic anhydride, acetic acid and boron trifluoride into 2,3,4,5,6-pentaacetoxytoluene (III). The action of dilute base upon the antibiotic produced a mixture of quinones, from which 2,3,5,6-tetraacetoxytoluene (IV) was obtained after reductive acetylation. A comparison of the n.m.r. spectra of terreic acid and 2,3-epoxy-1,4-naphthoquinone, together with other physical data and the chemical transformation products, allows the formulation of terreic acid as 5,6-epoxy-3-hydroxytoluquinone (I).

The isolation of the antibiotic terreic acid from culture broths of *Aspergillus terreus* was reported by Abraham and Florey[2] in 1949. Recently Kaplan, Hooper and Heinemann[3] obtained from cultures of an *Aspergillus* species an antibiotic which was demonstrated[4] to be identical with terreic acid. Although the antibiotic showed *in vitro* activity against gram-positive and gram-negative bacteria and fungi, *in vivo* tests were unpromising.[3] From a chemical standpoint, however, terreic acid is of interest because it possesses a combination of properties unusual for compounds of comparable molecular size. In this paper evidence is presented for the formulation of terreic acid as 5,6-epoxy-3-hydroxytoluquinone (I).

Terreic acid, $C_7H_6O_4$, may be purified either by sublimation or crystallization to give pale yellow

(1) (a) National Institutes of Health Postdoctoral Fellow, 1956–1957; (b) Bristol Laboratories Fellow, 1953–1954.

(2) E. P. Abraham and H. W. Florey, "Antibiotics," H. W. Florey, *et al.*, eds. Oxford University Press, New York, N. Y., 1949, Vol. I, p. 337. The discovery that *Aspergillus terreus* produces an antibiotic substance was made by W. H. Wilkins and G. C. M. Harris, *Brit. J. Exp. Path.*, **23**, 166 (1942).

(3) M. A. Kaplan, I. R. Hooper and B. Heinemann, *Antibiotics & Chemotherapy*, **4**, 746 (1954).

(4) Comparisons were made by Dr. K. R. Henery-Logan of this laboratory with the last few existing milligrams of authentic terreic acid, which were very kindly supplied by Professor Sir Howard Florey of Oxford. Terreic acid from Bristol Laboratories had m.p. 127–127.5°, while that from Oxford had m.p. 120–121°, but a mixed m.p. of 126.5–127° indicated that the two samples were identical, as did comparisons of their infrared and ultraviolet spectra.