Partial Methylation of Quercetin: Direct Synthesis of Tamarixetin, Ombuin and Ayanin

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Methylation of the pentahydroxy flavone, quercetin, was observed to proceed in distinct stages. From the reaction mixture (methyl sulfate/potassium carbonate/acetone), the isolation of three major intermediates, a mono-, a di- and a trimethyl ether and their identification as the naturally occurring ethers: tamarixetin, ombuin and ayanin is described.

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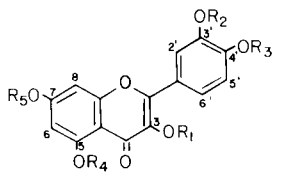
Methylation of flavonoid compounds has been the subject of active study for over a half-century, because: a) the methyl ethers serve well for the characterization of the highly polar hydroxy flavones; b) they are intermediates in the synthesis; and c) numerous partial and complete methylation products occur in nature. It was known that the hydroxyl function at 5, which is protected by hydrogen-bonding, resisted methylation by diazomethane or methyl iodide/base but was methylated by methyl sulfate/base or, preferably, methyl sulfate/potassium carbonate/acetone (1,2). No such selectivity was observed with the hydroxyl groups at the other positions until Simpson and Benton (3) reported that, in the presence of aqueous alcoholic sodium carbonate and methyl sulfate, the rates of methylation followed the sequence $3 > 3' > 4' > 7$. However, when bicarbonate was substituted, they observed the exactly opposite sequence. No actual products were isolated, the evidence being based mostly on uv spectral data.

We needed a quantity of 3,3′,4′,7-tetra-*O*-methylquercetin for a synthetic program and, during the study for a practical method, we observed that the methylation of quercetin appeared to proceed in distinct stages. This paper describes the isolation and characterization of the three major intermediates: the mono, di- and tri-*O*-methylquercetins which were identified as the naturally occurring ethers of quercetin: tamarixetin (**1**), ombuin (**2**) and ayanin (**3**), respectively.



1. $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = CH_3$
2. $R_1 = R_2 = R_4 = H$, $R_3 = R_5 = CH_3$
3. $R_2 = R_4 = H$, $R_1 = R_3 = R_5 = CH_3$
4. $R_4 = H$, $R_1 = R_2 = R_3 = R_5 = CH_3$

Preliminary experiments in the system: acetone/methyl sulfate (4 eq)/potassium carbonate (4 eq) showed that in 5 hours, the reaction gave the tetra- and penta-*O*-methylquercetins in the ratio 5:1. However, a tlc-examination during the reaction (Figure 1) showed two other spots (Rf 0.4 and 0.6) which progressively decreased. Relative proportions of these intermediates could be increased when 2 or 3 equivalents each of the reagents were used.

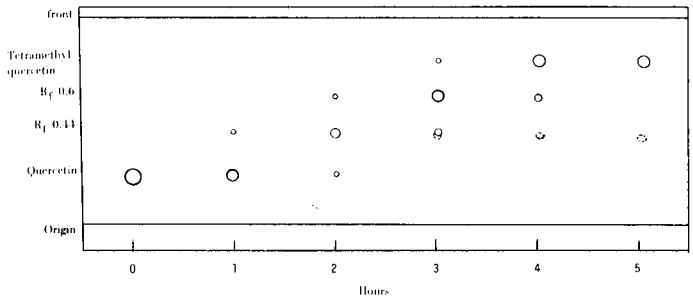


Figure 1. Tlc of Methylation Products of Quercetin. [Key: ○ - yellow; ◌ - blue fluorescence due to pentamethylquercetin]. Solvent system: 20% acetone in benzene.

In addition, the spot of Rf 0.6 was found to be a mixture of two components, one being the major when 3-4 equivalents of reagent were used and the other when 2-3 equivalents were used.

The three intermediates (one of Rf 0.4 and two of Rf 0.6) were separated on the basis of: a) partition between benzene and aqueous sodium carbonate, b) formation of a lead complex and c) chromatography on Florisil (see Experimental). Preliminary nmr spectral data showed that the component (Rf 0.4) was the monomethyl ether **1**, and the two components with Rf 0.6 as the di- and trimethyl

Table I

Nmr Signals of the Aromatic Protons in Quercetin and Derivatives and Their Shifts Due to Acetylation

Compound	Chemical Shifts of Protons (Hz) and Their Displacement									
	H_8	Δ	H_6	Δ	H_5'	Δ	H_2'	Δ	H_6'	Δ
1. Quercetin (a)	388		374		416		465		457	
2. Pentaacetylquercetin (b)	411	+23	437	+63	438	+22	461	-4	460	+3
3. Mono-*O*-methylquercetin (a) **1**	386		374		424		463		461	
4. Acetate of **1** (b)	411	+25	439	+65	422	-2	453	-10	463	+2
5. Di-*O*-Methylquercetin (a) **2**	400		379		425		462		465	
6. Acetate of **2** (b)	397	-3	411	+32	423	-2	454	-8	464	-1
7. Tri-*O*-methylquercetin (a) **3**	396		378		424		455		445	
8. Acetate of **3** (b)	394	-2	407	+29	422	-2	467	+12	479	+34
9. Tetra-*O*-methylquercetin (b) **4**	386		381		419		460		465	
10. Acetate of **4** (b)	395	+9	408	+27	418	-1	458	-2	462	-3
11. Penta-*O*-methylquercetin (b)	381		390		418		459		463	

Solvents used: (a) Dimethyl-d_6-sulfoxide. (b) Deuteriochloroform.

ethers **2** and **3**. In each, the 5-OH was free as indicated by the observed low field signal for the H-bonded hydroxyl in the nmr spectra.

Mono-*O*-methylquercetin (**1**).

A comparison of the melting points of the four possible monomethyl ethers and those of their acetates indicated that **1** might be the 4′-*O*-methyl ether. Although its uv spectral behavior resembled that of the 4′- as well as the 7′-*O*-methyl ether, shifts induced by sodium acetate (4) clearly indicated that the 7-hydroxyl group was free. Similarly, the magnitude of shift induced by boric acid (10-12 nm) in comparison with that from quercetin (20-24 nm) indicated that either the 3′ or the 4′-hydroxyl was methylated (4).

A more conclusive proof for the 4′-ether was obtained from the nmr spectral data in accordance with published figures on the effects of methylation (5). A comparison of the signals of **1** and of quercetin showed a shift for H_5' of +8 Hz and for H_2' of -2 Hz (Table I. A positive shift is a downfield shift.) Although this would not distinguish between the 3′ and the 4′-ether, a comparison of the spectra of **1** and of its acetate with those of quercetin and its acetate (Table I, Δ values) gave a convincing proof for the 4′-ether structure. The signal of H_5' was displaced by +24 while H_2' by +6. Thus, **1** was shown to be the 4′-methyl ether, tamarixetin (6).

Di-*O*-methylquercetin (**2**).

Of the six possible dimethyl ethers: 3,3′; 3,4′; 3,7; 3′,4′; 3′,7 and 4′,7, comparison of the physical and uv spectral data suggested that **2** might be the 4′,7-dimethyl ether. Nmr spectral comparison of **2** and quercetin showed the following shifts due to methylation: H_8, +12 Hz; H_6, +5 Hz; H_5', +9 Hz, H_2', -3 Hz and H_6', +8 Hz. Methylation of the 7 hydroxyl group was clearly indicated. The location of 4′ for the second methoxyl was deduced from a comparison with the spectrum of its acetate and with that of quercetin and its acetate. The H_5' in ring B, showed the largest shift. The structure of **2** as the 4′,7-dimethyl ether, ombuin is thus assigned (7).

Tri-*O*-Methylquercetin (**3**).

With the 5 hydroxyl group being free, there were four possibilities: 3,3′,4′; 3,3′,7; 3,4′,7 and 3′,4′,7 for the trimethyl ether **3**. A choice between the 3,3′,7 and 3,4′,7 was indicated by the physical and uv spectral data. In addition to the evidence for 4′ and 7 methoxyl groups as seen before, significant upfield shifts for H_2' and H_6' suggested methylation of the 3 hydroxyl group. This was further supported by comparison with the spectrum of the acetate and with those of the pair: tetra-*O*-methylquercetin (**4**) and its acetate. Absence of shifts for H_2' and H_6' in **4** *vs.* its acetate, in contrast to the shifts seen in **3** and its acetate, clearly indicate that the 3′ hydroxyl group was free in **3**. Thus, the 3,4′,7-trimethyl ether structure was assigend to **3**, identical with that given for ayanin (8).

Figure 1 and the isolation of **1**, **2** and **3** show that the methylation of this pentahydroxyflavone proceeds in steps, with a sequence of methylation rates: $4' > 7 > 3 > 3' > 5$ being indicated. Although this sequence differs from that shown by Simpson and Benton (3), the present conclusions are based on actual isolation and determination of structures. Whether this reflects the respective acidities of the hydroxyl groups must be established with other examples. It is interesting that this sequence is also reflected by the occurrence of these three ethers in nature.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns apparatus

and were uncorrected. The following instruments were used for the spectra described here: Beckman DB (uv) and Varian A60A with tetramethylsilane as internal standard (nmr). Thin-layer chromatography was carried out using Merck Silica gel HF 254 + 366 on micro slides. The solvent system was 10-20% acetone in benzene.

Methylation.

A solution of quercetin (3 g.) in acetone (100 ml.) was boiled under reflux with methyl sulfate (1.95 ml., 2 equivalents) and potassium carbonate (2.8 g., 2 equivalents) for approximately 2½ hours and then diluted with water (100 ml.). The acetone was concentrated and the solid filtered. It was partitioned between benzene and 5% potassium carbonate in a countercurrent fashion. The aqueous layers were adjusted to *p*H 8 and extracted with ethyl acetate. Concentration of the extract and crystallization from ether, saturated with water, gave the mono-*O*-methylquercetin **1**, yield, 0.4 g., m.p. 248-250° (Lit. 6, m.p. 251-252°).

Anal. Calcd. for $C_{16}H_{12}O_7$: C, 60.76; H, 3.82. Found: C, 60.61; H, 3.58.

The above experiment was repeated with 2 equivalents of the reagents. After 3 hours, filtration, concentration and digestion with ethanol (10 ml.), the solid was filtered. The filtrate was treated with aqueous lead acetate 5% until no further precipitation occurred. The precipitate was filtered, stirred with dilute sulfuric acid (100 ml., 1*N*) and extracted with ethyl acetate. The extract was concentrated and the solid **2**, crystallized from methanol, yield 0.4 g., m.p. 231-233°. (Lit. 7, m.p. 229-230°).

Anal. Calcd. for $C_{17}H_{14}O_7$: C, 61.82; H, 4.27. Found: C, 62.02; H, 4.15.

The above experiment was repeated with 3 equivalents of methyl sulfate (2.9 ml.) and of potassium carbonate (4.2 g.) and the mixture refluxed for 3 hours. After filtration and concentration of the filtrate, the solid was digested with hot benzene (50 ml.) and filtered. The solid was taken up in 1:9 acetone-benzene (30 ml.) and applied to a column of Florisil (75 g.) in benzene. Elution with 5% acetone in benzene gave the major band from which the product **3** was recovered and crystallized from benzene, yield, 1.2 g., m.p. 173-175° (Lit. 8, m.p. 172-173°).

Anal. Calcd. for $C_{18}H_{16}O_7$: C, 62.79; H, 4.68. Found: C, 63.01; H, 4.86.

Acetylation.

Each of the above samples (0.1 g.) in pyridine (0.2 ml.) and acetic anhydride (1 ml.) was heated at 100° for 2 hours. Dilution with water, filtration and crystallization from methanol gave the respective acetates.

Acetate of **1**.

This compound had m.p. 199-201°. (Lit. 6, m.p. 201-202°).

Acetate of **2**.

This compound had m.p. 210-212°. (Lit. 7, m.p. 212-213°).

Acetate of **3**.

This compound had m.p. 176-177°. (Lit. 8, m.p. 176-177°).

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