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# Studies on Flavonol Degradation by Peroxidase (Donor: $H_2O_2$-oxidoreductase, EC 1.11.1.7): Part 2—Quercetin*

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*ABSTRACT*

*The peroxidatic transformation of quercetin was studied at pH 5·5 under UV-spectrophotometric control. The reaction products obtained after 3 minutes' incubation of quercetin (dissolved in ethylene glycol monomethyl ether) with peroxidase (Boehringer, Mannheim)/$H_2O_2$ were fractionated on preparative scale by Sephadex LH-20 liquid chromatography using methanol as solvent. More than twenty compounds could be detected by analytical HPLC, from which the following substances were characterized by their spectroscopic data (UV; IR; $^1$H- and, in part, $^{13}$C-NMR; EI- and, in part, FAB- and FD-MS): 2,4,6-trihydroxybenzoic acid* (Q1)*; 3,4-dihydroxybenzoic acid* (Q2)*; methyl 2,4,6-trihydroxyphenylglyoxylate* (Q3)*; 2-(3,4-dihydroxyphenyl)-2-hydro-3,5,7-trihydroxy-3-methoxy-4H-1-benzopyran-4-one (2 isomers)* (Q4)*; 2-(3,4-benzochinoyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one ('quercetinchinone')* (Q5)*; 2,3-epoxy-2-(3,4-dihydroxyphenyl)-3-[4O-[2-(3-hydroxyphenyl)-3,5,7-]trihydroxy-4H-1-benzopyran-4-onyl]-5,7-dihydroxy-4H-1-benzopyran-4-one (2 isomers)* (Q7) *and a trimer* (Q8) *structurally related to* Q7.

## INTRODUCTION

Some years ago, Noguchi & Mori (1969) reported on a plant enzyme degrading flavonoids in the presence of $H_2O_2$. Using rutin as substrate,

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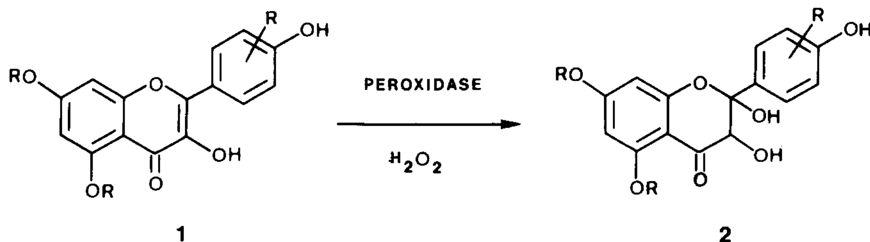
the authors found 3,4-dihydroxybenzoic- and 2,4,6-trihydroxybenzoic acid as products of peroxidase catalysis. In a series of investigations on flavonoid degradation Barz (1975*a*; 1975*b*; 1977; 1981) demonstrated that, after removal of the various glycosidic moieties, not only flavonols, but also chalcones, flavanols, phenolic acids and aurones can be used as substrates by peroxidase. According to Frey-Schröder & Barz (1979), flavonols (*1*) are degraded via 2,3-dihydroxyflavanones (*2*). For this enzymic degradation, the hydroxyl functions in positions 3 and 4′ must be free (Scheme 1).




**Scheme 1.**

Furthermore, it could be shown that the intermediates (*2*) are enzymatically degraded with the side chain phenyl rings being converted to substituted benzoic acids. These acids can be further transformed by hydroxylation, decarboxylation and ring fission reactions (Daly & Jerina, 1970; Berlin & Barz, 1975; Patzlaff & Barz, 1978). The biological significance of such complex cascades of peroxidatic degradation reactions has still to be elucidated; various aspects have been critically discussed by Barz & Köster (1981).

In spite of the progress achieved, more intensive investigations are required to elucidate the degradation pathways of flavonoids. In our studies, we investigated the peroxidatic degradation of flavonols in model systems with the aim of structural elucidation of reaction products formed. In a previous paper (Miller & Schreier, 1985) we reported on the peroxidatic kaempferol degradation; in this paper, our results, obtained using the model system quercetin/$H_2O_2$/peroxidase, are described.

## MATERIALS AND METHODS

### Materials

Quercetin was from Roth, Karlsruhe, and peroxidase (purity grade II) from Boehringer, Mannheim.

## Standard incubations

*UV-spectrophotometric assay*

Fifty microlitres of quercetin solution (2 mM/litre in ethylene glycol monomethyl ether) and 50 μl of aqueous $H_2O_2$ solution (4 mM/litre) were added to 1·85 ml 0·1 M citrate–phosphate buffer (pH 5·5). The reaction was started using 50 μl of peroxidase solution (1 mg/ml in citrate–phosphate buffer, pH 5·5). The reaction was photometrically followed (220–400 nm; 20 °C) for at least 20 min. Reference measurements were performed without quercetin addition.

*Large-scale incubations*

Twenty-five millilitres of aqueous $H_2O_2$ solution (100 mM/litre) and 10 ml of peroxidase solution (0·2 mg/ml) were added to 500 ml 0·1 M citrate–phosphate buffer (pH 5·5). The reaction was started with 50 ml of quercetin solution (20 mM/litre in ethylene glycol monomethyl ether) at 20 °C. After 3 min, the reaction was stopped by the addition of 100 mg $NaN_3$. After acidification with HCl (5 %) to pH 2·0 the mixture was extracted immediately with diethyl ether (5 × 100 ml). The extracts were combined, dried over $Na_2SO_4$ and carefully concentrated using a Vigreux column.

## Liquid chromatographic separations

*Analytical HPLC*

A Kontron LC 720 apparatus equipped with a 5 × 100 mm Spheri-5 column (RP-18, 5 μ) and an Uvikon 720 detector (Kontron) were used. Measurements were carried out at 295 nm employing a non-linear gradient of acetic acid (5 %)/methanol (Miller & Schreier, 1985). The flow rate was 1 ml/min and 20 μl injections were applied.

*Liquid chromatography on Sephadex LH-20* (cf. *Fig. 2*)

The concentrated diethyl ether extract (0·2 ml) of reaction products of peroxidatic quercetin degradation was added onto a Sephadex LH-20 column (1·5 × 60 cm) and, in the first step, fractionated into 64 fractions (1 ml) eluting with methanol (flow rate, 30 ml/h). Rechromatography of combined (and concentrated) fractions 1–15, 38–41, 58–62 and 63–64 resulted in the separation of *Q1–Q3*, *Q5* and *Q7* and *Q8*, respectively. From combined and concentrated fractions 30–37 and 52–57, respectively, new eluates were isolated by rechromatography which were again

separated on Sephadex LH-20 yielding *Q4* and *Q6*, respectively. At each step, the individual eluates were checked by analytical HPLC.

## Identification of reaction products

*UV-spectroscopy*
UV-spectrophotometric measurements were carried out using a Zeiss PMQ II spectrophotometer and different solvents, as well as shift reagents, as described by Markham (1982).

*IR-spectroscopy*
IR spectroscopic measurements were performed in KBr employing a Beckman IR 4240 IR spectrophotometer (400–4000 $cm^{-1}$).

*Mass spectrometry*
EI mass spectra were recorded using a Finnigan-MAT 44 mass spectrometer at 70 eV and 250 °C. FAB-MS measurements were carried out employing a Finnigan-MAT 312 ('FAB-gun'; 7 kV Xe; methanol/acetic acid/glycerol; 80 °C). FD-MS spectra were recorded with a Finnigan-MAT 312 apparatus equipped with an FD source using the 'emitter dipping-technique' (Geiger & Schwinger, 1980).

*$^{1}$H-NMR spectroscopy*
400 MHz $^{1}$H-NMR spectra were measured at 20 °C using a Bruker Cryospec WM 400 apparatus, acetone-$d_6$ as solvent and TMS as internal standard.

*$^{13}$C-NMR spectroscopy*
The measurements of $^{13}$C-NMR spectra were performed at 20 °C using a Bruker Cryospec WM 400 apparatus, acetone-$d_6$ as solvent and TMS as internal standard.

## Analytical data of isolated reaction products

*Q1:* 2,4,6-Trihydroxybenzoic acid. $C_7H_6O_5$ (170). EI-MS (43-68-125-52-51-69-126-123).

*Q2:* 3,4-Dihydroxybenzoic acid. $C_7H_6O_4$ (154). EI-MS (154-137-109-81-138-53).

*Q3:* Methyl 2,4,6-trihydroxyphenylglyoxylate. $C_9H_8O_6$ (212). UV ($CH_3OH$): $\lambda_{max}$ 292 nm (log $\varepsilon = 4{\cdot}1$); 260 nm (log $\varepsilon = 3{\cdot}6$). IR (KBr): 1740 $cm^{-1}$. EI-MS (137-153-152-57-59-109).

*Q4:* 2-(3,4-Dihydroxyphenyl)-2-hydro-3,5,7-trihydroxy-3-methoxy-4H-1-benzopyran-4-one (2 isomers). $C_{16}H_{14}O_8$ (334). UV ($CH_3OH$): $\lambda_{max}$ 290 nm (log $\varepsilon = 4{\cdot}0$). IR (KBr): 1640, 1620, 1460, 1300, 1170 $cm^{-1}$. $^1H$-NMR (400 MHz, acetone-$d_6$, TMS): $\delta = 5{\cdot}95$ (J = 2·2 Hz; H-6); 6·05 (J = 2·2 Hz; H-8); 7·04 (J = 2·2 Hz; H-2′); 6·7 (J = 8·5 Hz; H-5′); 6·84 (J = 2·2 Hz; H-6′); 5·25 (s); 5·5 (s) (H-2); 6·35 (s); 6·60 (s) (OH-3); 2·95 (s); 3·3 (s) (-$OCH_3$) ppm. EI-MS (301-300-167-168-316).

*Q5:* 2-(3,4-Benzochinoyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one ('Quercetinchinone'). $C_{15}H_8O_7$ (300). UV ($CH_3OH$): $\lambda_{max}$ 295 nm (log $\varepsilon = 4{\cdot}04$); 295 nm (log $\varepsilon = 3{\cdot}97$); 365 nm (log $\varepsilon = 3{\cdot}3$). Vis ($CH_3OH$): $\lambda_{max}$ 505 nm (log $\varepsilon = 2{\cdot}96$). IR (KBr): 1650 $cm^{-1}$. $^1H$-NMR (400 MHz, acetone-$d_6$, TMS): $\delta = 6{\cdot}16$ (J = 2·2 Hz; H-6); 6·24 (J = 2·2 Hz; H-8); 7·61 (J = 2·1 Hz; H-2′); 6·89 (J = 8·7 Hz; H-5′); 7·57 (J = 8·7; 1Hz; H-6′) ppm. EI-MS (301-302-300-158).

*Q6:* FAB-MS (455 $[M + H]^+$).

*Q7:* 2,3-Epoxy-2-(3,4-dihydroxyphenyl)-3-[4O-[2-(3-hydroxyphenyl)-3,5,7-]trihydroxy-4H-1-benzopyran-4-onyl]-5,7-dihydroxy-4H-1-benzopyran-4-one (2 isomers). $C_{30}H_{18}O_{14}$ (602).UV ($CH_3OH$): $\lambda_{max} = 305$ nm (log $\varepsilon = 4{\cdot}06$); 365 nm (log $\varepsilon = 4{\cdot}0$). IR (KBr): 1660, 1500 $cm^{-1}$. $^1H$-NMR (400 MHz, acetone-$d_6$, TMS): $\delta = 2$ A-rings with each 6·06 (J = 2·1 Hz; H-6); 6·06 (J = 2·1 Hz; H-8); 2 A-rings with each 6·25 (J = 2·0 Hz; H-6); 6·6 (J = 2·0; H-8); 2 B-rings with each 6·85 (J = 8·5 Hz; H-5′); 7·19 (J = 2·2; 8·5 Hz; H-6′); 7·38 (J = 2·2 Hz; H-2′); B-ring with 7·19 (J = 8·7 Hz; H-5′); 8·00 (J = 2·1, 8·7 Hz; H-6′); 7·90 (J = 2·1 Hz; H-2′); B-ring with 8·05 (J = 2·1 Hz; H-2′); 7·30 (J = 8·8 Hz; H-5′); 8·0 (J = 2·1, 8·7 Hz; H-6′) ppm. $^{13}C$-NMR (acetone-$d_6$, TMS): $\delta =$ 'I': 123 (C1′); 115 (C2′); 143 (C3′); 141 (C4′); 116 (C5′); 121 (C6′); 145 (C2); 137 (C2); 176 (C4); 160 (C5); 99 (C6); 164 (C7); 94 (C8); 157 (C9); 104 (C10). 'II': 126 (C1′); 117 (C2′); 145 (C3′); 147 (C4′); 118 (C5′); 123 (C6′); 91 (C2); 101 (C3); 188 (C4); 165 (C5); 97 (C6); 169 (C7); 98 (C8); 162 (C9); 100 (C10) ppm. EI-MS: 301-302-300. FD-MS: 303-302-301-601-602 $[M]^+$. FAB-MS: negative ions: 300-601 $[M-H]^-$; positive ions: 303-302-304-603 $[M + H]^+$. Determined: C 58·0; H 3·3; O 38·7. Found: C 57·6; H 4·0; O 38·6.

*Q8:* Trimer. $C_{45}H_{26}O_{21}$ (902). UV ($CH_3OH$): $\lambda_{max}$ 270 nm (log $\varepsilon = 4{\cdot}06$); 305 nm (log $\varepsilon = 4{\cdot}02$). IR (KBr): 1610, 1500 $cm^{-1}$. $^1$H-NMR (400 MHz, acetone-$d_6$, TMS): $\delta$ = A-ring I: 6·00 (J = 2·0 Hz; H-6); 6·04 (J = 2·0 Hz; H-8); A-ring II: 6·04 (J = 1·85 Hz; H-6); 6·14 (J = 1·85 Hz; H-8); A-ring III: 6·29 (J = 2·1 Hz; H-6); 6·62 (J = 2·1 Hz; H-8); B-ring I: 7·31 (J = 2·2 Hz; H-2′); 6·79 (J = 8·45 Hz; H-5′); 7·12 (J = 2·2, 8·45 Hz; H-6′); B-ring II: 7·46 (J = 2·2 Hz; H-2′); 7·03 (J = 8·6 Hz; H-5′); 7·60 (J = 2·2, 8·6 Hz; H-6′); B-ring III: 8·07 (J = 2·0 Hz; H-2′); 7·19 (8·6 Hz; H-5′); 7·99 (J = 2·0, 8·6 Hz; H-6′). EI-MS (301-302-300); FAB-MS (303-603-903 $[M + H]^+$). Determined: C 59·9; H 2·9; O 37·2. Found: 59·4; H 3·3; O 36·8.

## RESULTS AND DISCUSSION

### UV-spectrophotometric control of peroxidatic reaction

After incubation of quercetin with $H_2O_2$ and peroxidase at pH 5·5 the flavonol degradation was measured by the decrease of UV-absorption. As

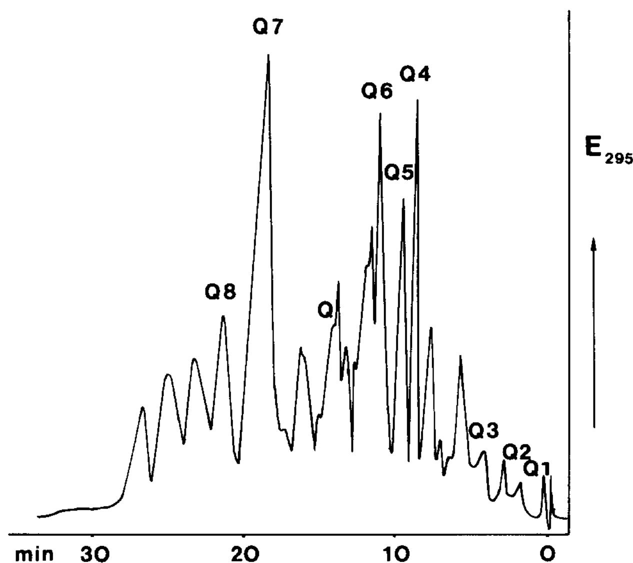



**Fig. 1.** HPLC separation (Spheri-5 column, 5 $\mu$) of reaction products (*Q1–Q8*) extracted by diethyl ether from quercetin (*Q*)/$H_2O_2$/peroxidase. Kontron LC 720 with Uvikon 720. Non-linear gradient (acetic acid (5 %)/methanol, *cf.* Miller & Schreier, 1985). Flow rate, 1 ml/min. Detection, 295 nm. Injection, 20 $\mu$l.

already observed using kaempferol as substrate (Miller & Schreier, 1985), the characteristic absorptions of the educt at 260 and 370 nm decreased rapidly and a pronounced UV-maximum at 300 nm was detected.

## Preparative isolation of reaction products

Preparative experiments were performed with quercetin (dissolved in ethylene glycol monomethyl ether)/$H_2O_2$/peroxidase at pH 5·5 (3 minutes incubation). The reaction products obtained by diethyl ether extraction could be separated into more than twenty individual components by analytical HPLC (Fig. 1) but attempts made to use HPLC on a preparative scale failed due to strong loss of resolution capacity. As already described in the previous paper (Miller & Schreier, 1985), liquid chromatography on Sephadex LH-20 was found to be a suitable preparative separation technique. The separation scheme developed is outlined in Fig. 2. The isolated compounds *Q1*–Q8 were checked by analytical HPLC; with the exception of *Q6*, in each case, only one peak was detected.

## Characterization of reaction products

In the following, the structures of the isolated products *Q1*–Q8 are discussed by means of their spectroscopic data recorded (see 'Materials and Methods' section).

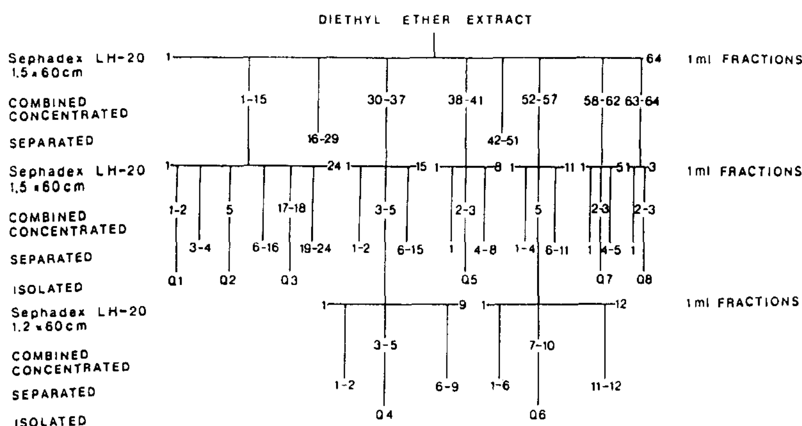


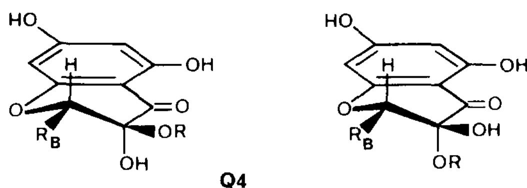

Fig. 2. Scheme of separation steps on Sephadex LH-20 used for preparative isolation of reaction products from quercetin/$H_2O_2$/peroxidase.

*Q1:* Less than 1 mg of *Q1* was isolated. It was identified as 2,4,6-trihydroxybenzoic acid by comparison of its gas chromatographic retention (as methyl ester) and EI-MS data with those of an authentic reference compound.

*Q2:* About 1 mg of *Q2* was obtained and was identified as 3,4-dihydroxybenzoic acid comparing its gas chromatographic retention (as methyl ester) and EI-MS data with those of an authentic reference sample.

*Q3:* About 1 mg of *Q3* was isolated. It was identified as methyl 2,4,6-trihydroxyphenylglyoxylate comparing its spectroscopic data with those of an authentic reference compound.

*Q4:* About 4 mg of *Q4* was obtained. First of all, a structural relationship to dihydroquercetin was indicated by its UV-spectrum. This indication was supported by the $^1$H-NMR spectrum. However, compared with dihydroquercetin, the $^1$H-NMR- and EI-MS data pointed out an additional substitution at C-3 (-$OCH_3$). Presupposing two isomer forms for *Q4* ($^1$H-NMR), the structures outlined in Scheme 2 ($R = CH_3$; $R_B$ = B-ring) are proposed.




**Scheme 2.**

Some years ago, Hauteville *et al.* (1979) provided detailed information including spectroscopic data about stereoisomers of this type.

*Q5:* The violet reaction product, from which about 5 mg could be isolated, was identified as 2-(3,4-benzochinoyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one comparing its chromatographic (HPLC) and spectroscopic data with those of a sample of 'quercetinchinone' obtained by chemical oxidation of quercetin with $PbO_2$ (Loth & Diedrich, 1968).

*Q6:* This product could not be obtained in pure form. The FAB-MS data provided a molecular mass of 454.

**TABLE 1**
UV-Spectroscopic Data (nm) of *Q7* and Quercetin (*Q*) Using Various Solvents and Shift Reagents (Markham, 1982)

| | $CH_3OH$ | $NaOCH_3$ | $NaOAc$ | $H_3BO_3$ | $AlCl_3$ | $AlCl_3/HCl$ |
|---|---|---|---|---|---|---|
| *Q7* | 270 | 275 | 275 | 295 | 260 | 260 |
| | 305 | 335 | 335 | 365 | 310 | 310 |
| | 365 | 405 | 395 | 440 s | 355 | 355 |
| | — | — | — | — | 420 | 420 |
| *Q* | 255 | 247 s | 257 s | 261 | 272 | 265 |
| | 269 s | 321 d | 274 | 303 s | 304 s | 301 s |
| | 301 s | — | 329 | 388 | 333 | 359 |
| | 370 | — | 390 d | — | 458 | 428 |

s = shoulder; d = decomposition.

*Q7:* It was the main component among the reaction products (*cf.* Fig. 2) of peroxidatic quercetin degradation; about 50 mg of pure *Q7* could be isolated. The UV data measured using different shift reagents (Markham, 1982) are outlined in Table 1. In the '$NaOCH_3$' spectrum a distinct bathochromic shift of the UV maxima was observed, indicating free 5,7-dihydroxy functions in *Q7*. Nearly identical '$AlCl_3$' spectra were observed; they do not correspond to the UV spectrum measured in methanol. As acid-stable complexes were formed between hydroxyl and neighbouring ketone functions, the quercetin structures at C-4 and C-5 should be present unchanged in *Q7*. Furthermore, the UV maximum at 305 nm indicates a dihydroquercetin structure.

The $^1$H-NMR spectrum of *Q7* outlined in Figs 3 and 4 demonstrates that the aromatic substitution pattern of A- and B-rings of quercetin remained unchanged (*cf.* Fig. 4, A1A; A1B; A2A; A2B; B1A; B2A; B2B). Some signals indicate that *Q7* consists of two substances showing nearly identical structures. On the one hand, for example, closely related signals with comparable coupling constants are observed (e.g. 6·25 ppm; J = 2·0 Hz; 6·60 ppm; J = 2·0 Hz); on the other hand, some signals can be detected showing integration equivalents, which correspond to the addition of each of the closely related signals. For instance, the integration of the signal at 6·06 ppm (J = 2·1 Hz) corresponds with that of the two signals at 6·25 (J = 2·0 Hz) and 6·60 ppm (J = 2·0 Hz). Thus, it can

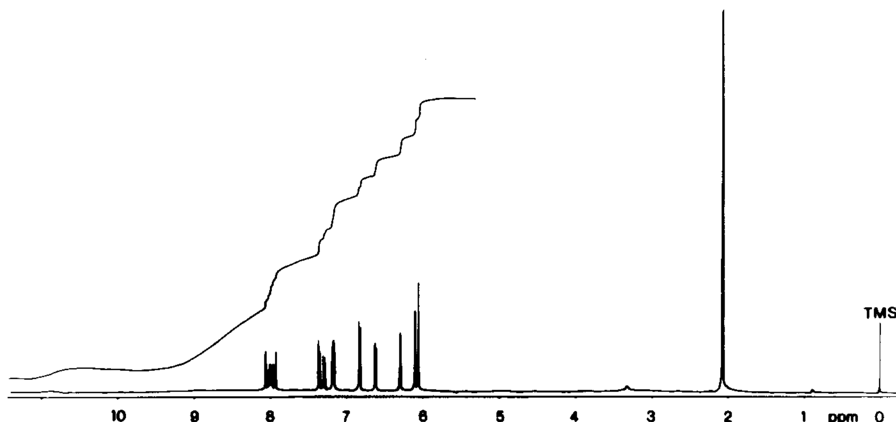



**Fig. 3.** 400 MHz $^1$H-NMR spectrum (acetone-$d_6$, TMS) of *Q7*.

be supposed that the A-ring *meta*-signals of two nearly identical substances coincide at 6·06 ppm (J = 2·1 Hz).

On the basis of this concept, the signals of the two A-rings at 6·06 ppm (J = 2·1 Hz; H-6, H-8) and two further A-rings at 6·25 ppm (J = 2·0 Hz; H-6) and 6·60 ppm (J = 2·0 Hz; H-8) are detectable. The B-ring signals are found for two B-rings at 6·85 ppm (J = 8·5 Hz; H-5′), 7·19 ppm (J = 2·2, 8·5 Hz; H-6′) and 7·38 ppm (J = 2·2 Hz; H-2′), respectively, as well as two further B-rings at 7·19 ppm (J = 8·7 Hz; H-5′), 8·00 ppm (J = 2·1, 8·7; H-6′), 7·90 ppm (J = 2·1 Hz; H-2′) and 8·05 ppm (J = 2·1 Hz; H-2′), 7·30 ppm (J = 8·8 Hz; H-5′) and 8·00 ppm (J = 2·1, 8·7 Hz; H-6′), respectively. In total, the signals of four A- and four B-rings result, showing aromatic substitution patterns identical to quercetin. As a molecular mass of 602 was determined by FAB- and FD-MS spectrometry, for *Q7* two nearly identical products, both exhibiting dimer structures, can be proposed.

In order to get an insight into the oxidation state of the C-ring, in Table 2 the $^1$H-NMR signals of aromatic protons of some flavonoids are compared with those of substructures A1A, A1B, B1A, and B1B, respectively (Fig. 4). From the data summarized in Table 2 the dependence of proton resonance of A- and B-rings on the oxidation state of C-ring is demonstrated. Compared with the data taken from the literature (Mabry *et al.*, 1970) it will be clear that the C-2/C-3 double bond (of the educt) does not exist in the above-mentioned *Q7* substructures. On the other hand, the signals at 6·25 ppm (J = 2·0 Hz) and 6·60 ppm (J = 2·0 Hz) (A2A, A2B in Fig. 4) are, together with the B-ring protons (B2A,

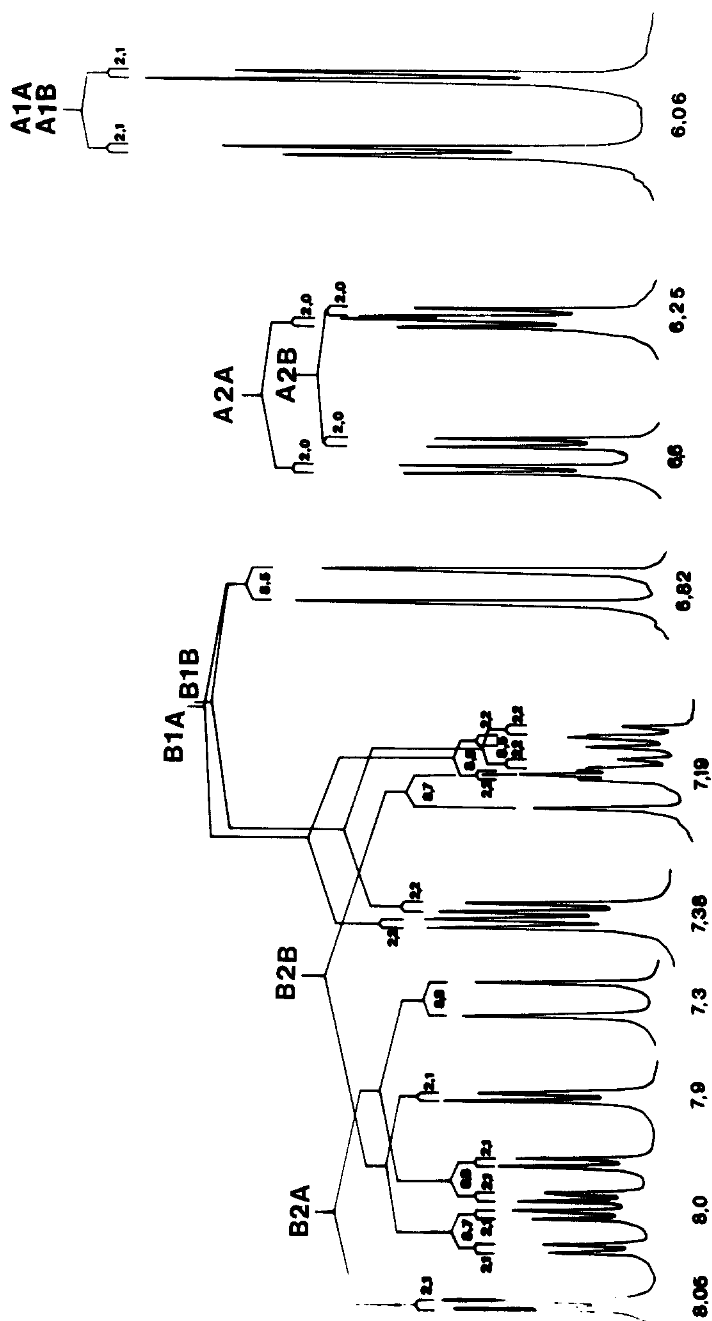



**Fig. 4.** Details of 400 MHz $^1$H-NMR spectrum (acetone-$d_6$, TMS) of *Q7*.

**TABLE 2**
$^{1}$H-NMR Shifts (ppm, TMS) of Some 5,7,3′,4′-Substituted Flavonoids (Mabry *et al.*, 1970) Compared with those of *Q7* Substructures A1A, A1B, B1A, B1B (*cf.* Fig. 4). Coupling Constants (Hz) in Parentheses

| | *H-6* | *H-8* | *H-2′* | *H-5′* | *H-6′* |
|---|---|---|---|---|---|
| A1A | 6·06 (2·1) | 6·06 (2·1) | — | — | — |
| A1B | 6·06 (2·1) | 6·06 (2·1) | — | — | — |
| B1A | — | — | 7·38 (2·2) | 6·85 (8·5) | 7·19 (2·2;8·5) |
| B1B | — | — | 7·38 (2·2) | 6·85 (8·5) | 7·19 (2·2;8·5) |
| Dihydroquercetin | 5·95 (2·0) | 6·05 (2·0) | 6·85 (2·1) | 6·85 (8·5) | 6·85 (2·1;8·5) |
| Hesperidin | 5·8 (2·0) | 6·0 (2·0) | 6·8 (2·1) | 6·85 (8·6) | 6·8 (2·1;8·6) |
| Luteolin | 6·15 (2·2) | 6·45 (2·2) | 7·3 (2·1) | 6·85 (8·6) | 7·3 (2·1;8·6 |
| Quercetin | 6·15 (2·2) | 6·45 (2·2) | 7·7 (2·2) | 6·85 (8·5) | 7·7 (2·2;8·5) |

B2B), downfield-shifted (Table 3), suggesting two substructures with C-2/C-3 double bond.

Thus, the data represented in Tables 2 and 3 demonstrate saturation at the C-2/C-3 position in the substructures A1A, A1B, B1A and B1B as well as unsaturation in structures A2A, A2B, B2A and B2B. As the two components in *Q7* are not present in the proportion 1:1 (*cf.* integration differences, Fig. 4) the monomer substructures A1A, B1A and A2A, B2A can be allied to a dimer 'I'. In a similar way, a dimer 'II' can be formed by the monomers A1B, B1B and A2B, B2B, respectively. Due to the maintenance of the aromatic substitution pattern of quercetin, the linkage between 'I' and 'II' has to be effected by a hydroxyl function. However, OH functions at C-5 and C-7 of unsaturated substructures have

**TABLE 3**
$^{1}$H-NMR Shifts (ppm, TMS) of *Q7* Substructures Containing C-2/C-3 Double Bond Compared with those of Quercetin (*Q*). Coupling Constants (Hz) in Parentheses

| | *H-6* | *H-8* | *H-2′* | *H-5′* | *H-6′* |
|---|---|---|---|---|---|
| A2A | 6·25 (2·0) | 6·60 (2·0) | — | — | — |
| A2B | 6·25 (2·0) | 6·60 (2·0) | — | — | — |
| B2A | — | — | 8·05 (2·1) | 7·3 (8·8) | 8·0 (2·1;8·8) |
| B2B | — | — | 7·9 (2·1) | 7·19 (8·7) | 8·0 (2·1;8·7) |
| *Q* | 6·15 (2·2) | 6·45 (2·2) | 7·7 (2·2) | 6·85 (8·5) | 7·7 (2·2;8·5) |

to be excluded, as otherwise the downfield-shifts of B-ring protons (B2A and B2B) cannot be explained.

In order to elucidate the substitution at C-2/C-3 of saturated substructures and to check the number of OH protons, a $^{1}$H-NMR spectrum with $D_2O$ exchange was recorded. Figure 5 shows that, after addition of $D_2O$, sixteen protons were exchanged, i.e. eight protons for each dimer. These results exclude OH substitution at the C-2/C-3 position of saturated substructures and indicate the formation of an ether bridge (less 2 × 2 OH functions) as linkage between the monomer substructures of both the molecules.

Supplementary to the $^{1}$H-NMR spectra, the $^{13}$C-NMR data (see 'Materials and Methods' section) indicate dimerization leading to a product with 30 C-atoms. As was done for the results of $^{1}$H-NMR spectroscopy (Tables 2 and 3), the $^{13}$C-NMR data of *Q7* were tried to relate to those of the educt. As outlined in Table 4, one monomer substructure ('I') shows, with the exception of the C-3'- and C-4' signals, resonances identical to those of the educt. The signals of the second monomer ('II'), however, differ distinctly from those of quercetin. The downfield position of C-4 in 'II' excludes conjugation between A- and B-rings; this fact is supported by the highfield-shift of C-2 and C-3 signals. The relative highfield-shift of C-2 (91 ppm) and C-3 (101 ppm) signals (quercetin: 146·9 and 135·6, respectively) indicates that, at these positions, substitution occurs, which can be only an oxygen function under the reaction conditions. The epoxide structure postulated by $^{1}$H-NMR data is supported by these $^{13}$C-NMR signals (Bremser *et al.*, 1982).

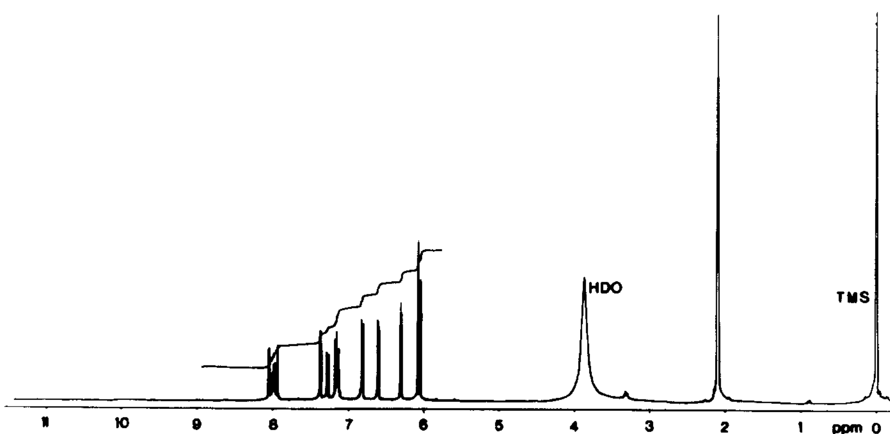



**Fig. 5.** 400 MHz $^{1}$H-NMR spectrum of *Q7* after addition of $D_2O$ (*cf.* Fig. 3).

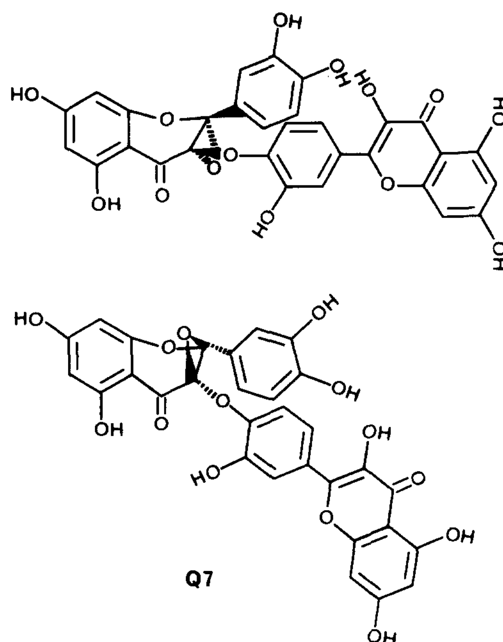
As the C-3 signal of monomer 'I' (137 ppm) was not changed compared with that of quercetin, there is, together with the $^{1}$H-NMR data, sufficient evidence for the linkage of monomers by the OH function at C-4′. A distinct differentiation of the postulated two stereoisomers ($^{1}$H-NMR) could not be achieved by $^{13}$C-NMR spectroscopy; only in parts were double signals found.

As to the structures in *Q7*, it can be summarized that two dimers, each with the molecular mass of 602 (FAB-MS, FD-MS) occur, in each of

**TABLE 4**
$^{13}$C-NMR Data (ppm, TMS) of *Q7* Substructures Compared with those of Quercetin (Harborne & Mabry, 1982)

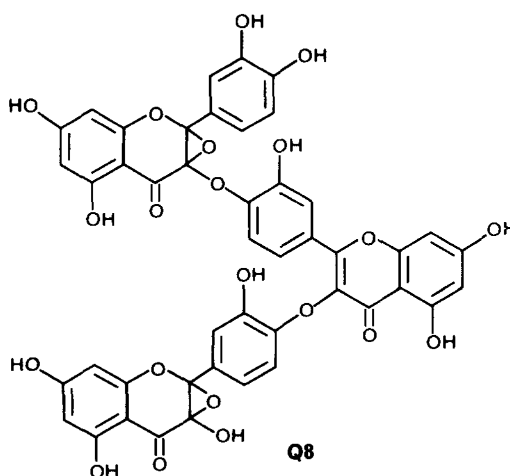
| | *Quercetin* | *Monomer 'I'* | *Monomer 'II'* |
|---|---|---|---|
| C-1′ | 122·0 | 123 | 126 |
| C-2′ | 115·3 | 115 | 117 |
| C-3′ | 145·0 | 143 | 145 |
| C-4′ | 147·6 | 141 | 147 |
| C-5′ | 115·6 | 116 | 118 |
| C-6′ | 120·0 | 121 | 123 |
| C-2 | 146·9 | 145 | 91 |
| C-3 | 135·6 | 137 | 101 |
| C-4 | 175·7 | 176 | 188 |
| C-5 | 160·7 | 160 | 165 |
| C-6 | 98·2 | 99 | 97 |
| C-7 | 163·9 | 164 | 169 |
| C-8 | 93·4 | 94 | 98 |
| C-9 | 156·2 | 157 | 162 |
| C-10 | 103·0 | 104 | 100 |

which an unchanged quercetin substructure is present. The second monomer substructures each show aromatic substitution patterns identical to the educt ($^{1}$H-NMR), but the proton resonances clearly demonstrate the saturation of the C-2/C-3 bond. The molecular mass of each of these substructures is 300 (FAB-MS; FD-MS) indicating, for the substitution at C-2/C-3, an oxygen in the form of an epoxide structure. From the resulting sp$^{3}$-hybridization at C-3, the formation of axial–equatorial structures as shown in Scheme 3 is possible.




**Scheme 3.**

*Q8:* About 10 mg of this product was isolated. The $^{1}$H-NMR spectrum indicates trimerization of quercetin (see 'Materials and Methods' section) without any change of the aromatic substitution pattern of the educt. The trimerization was confirmed by the FD-MS spectrum, providing a molecular mass of 902. Evaluating the $^{1}$H-NMR data, in *Q8* a dimer




**Scheme 4.**

structure identical to *Q7* can be proposed. Although the conjugation through the C-ring is lost, the B-ring signals of the third monomer substructure are, compared with those of the A-ring protons, downfield-shifted, indicating a linkage at the C-4 position by an ether bridge to the C-3 of quercetin substructure in the dimer. On the basis of a dimer substructure in *Q8* identical to *Q7*, the structure shown in Scheme 4 can be proposed for *Q8*.

The results obtained during this work confirm and supplement earlier findings of Barz (1977; 1981). One of the first steps in the peroxidatic transformation of flavonols consists of the attack at the olefinic C-2/C-3 bond leading to incorporation of oxygen into the flavonoid structure and, finally, to the formation of a very complex composition of polar reaction products. The studies of metabolism of phenolics are strongly complicated by oligo- and polymerisation reactions (Dieterman *et al.*, 1969; Hösel *et al.*, 1975), in which we could obtain a small insight by these model studies.

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