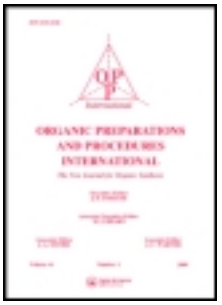

Organic Preparations and Procedures International: The New Journal for Organic Synthesis

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uopp20

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Version of record first published: 11 Feb 2009.

To cite this article: E. Kiehlmann (1999): PREPARATION AND PARTIAL DEACETYLATION OF DIHYDROQUERCETIN ACETATES, Organic Preparations and Procedures International: The New Journal for Organic Synthesis, 31:1, 87-97

To link to this article: http://dx.doi.org/10.1080/00304949909355676

ORGANIC PREPARATIONS AND PROCEDURES INT., 31 (1), 87-97 (1999)

PREPARATION AND PARTIAL DEACETYLATION OF DIHYDROQUERCETIN ACETATES

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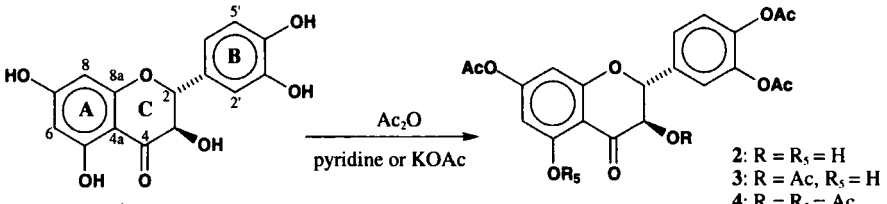
Flavanoids, the most widely distributed class of plant pigments, are attracting increasing interest for their remarkably large variety of physiological effects in plants and animals, *e. g.*, as antioxidants, phytoalexins, important dietary constituents, enzyme inhibitors and potential anticancer agents.[1] Among natural 3-acetoxyflavanones, pinobanksin 3-acetate is known for its antimicrobial activity[2] while the closely related dihydroquercetin 3-acetate (**7b**), recently isolated from a South American herb, has been found to be eighty times as sweet as sucrose.[3] The potential use of **7b** as artificial sweetener[4] and our interest in the chemistry of dihydroquercetin[5] prompted us to investigate the feasibility of synthesizing this monoacetate by partial acetylation. While enzyme-mediated protection/deprotection reactions of hydroxylated flavans[6] and flavones[7] have been shown to be regioselective, reactivity comparisons have not been reported for the hydroxyl groups of dihydroflavonols which exhibit similarly large acidity differences.[8] One should expect that the OH groups of a polyhydroxyflavanone are likewise acetylated sequentially and that the reaction can be controlled by appropriate variation of the experimental conditions. The success of such a project depends crucially on the availability of analytical techniques that permit the detection, separation and unambiguous identification of a large number of structurally similar reaction products. We have chosen the pentahydroxyflavanone (+)-*trans*-dihydroquercetin (**1**) as substrate for our studies because it is abundantly available from Douglas fir bark,[9] a major waste product of the forest industry of the Pacific Northwest. For this compound, one penta-, five isomeric tetra-, ten tri-, ten di- and five monoacetates (excluding stereoisomers) are possible but only three are presently known. Another twenty have now been obtained in our laboratory by acetylation of **1** and partial deacetylation of its pentaacetate.

Under the standard conditions employed for the derivatization of flavanoids, acetic anhydride converts dihydroquercetin (dhq) to its pentaacetate (*Scheme 1*) which has been reported to be noncrystallizable[10] and to melt sharply,[3,10-16] within 1-2° ranges, anywhere from 82° to 148°. Quantitative acetyl determination[11] and NMR spectroscopy[14,16,17] verify its structure as (+)-(2R,3R)-3,3',4',5,7-pentaacetoxyflavanone (**4**), and its optical activity[12-15] confirms retention of absolute configuration at the chiral centers.[5,16,18] More consistent melting points (147°-155°) have been obtained for the inactive

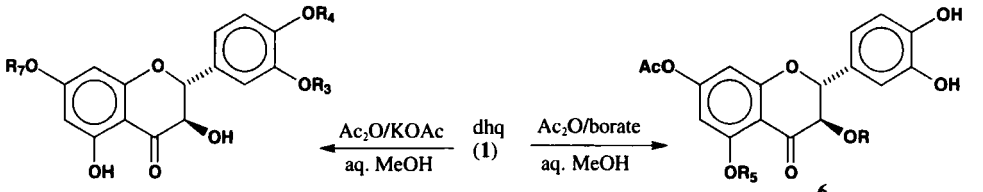


1

Scheme 1

product[11,12,17,19] derived from racemic starting material. Although the mp. discrepancies may be due to partial racemization,[12] our observation and analysis of tiny contaminant peaks in the proton NMR spectra of samples recrystallized from methanol indicate incomplete reaction and/or partial alcoholysis (see below) as major causes.

When the reaction is performed with catalytic amounts of pyridine or potassium acetate, *i. e.*, under conditions known to hinder or entirely prevent acetylation of the chelated 5-hydroxyl group of flavones,[20,21] dhq-3,3',4',7-tetraacetate (**3**)[11,12] is formed as major and **4** as minor product. Elemental analysis is not suitable for distinction between these two or any other partially acetylated dihydroquercetins because their calculated carbon and hydrogen percentages (58.37% and 4.31%, respectively, for the pentaacetate vs. 58.96% and 4.07% for a monoacetate) fall within the commonly accepted experimental error of ±0.3%. The low-field proton signal at δ 11.35 (chelated ArO**H**) and the high-field acetyl singlet at δ 2.00 (the methyl protons of aryl acetates resonate at δ 2.20-2.40) constitute unambiguous evidence for the position of the free OH-group at C-5, as originally proposed[12,23] on the basis of IR and UV spectral data. Our ^{1}H NMR data in $CDCl_3$ also agree with those previously reported[22] for **3** except for the chemical shifts of H-6 and H-8 (δ 6.39/6.33 vs. 6.79/6.61): the higher literature values are characteristic of **4** which appears to have formed as byproduct in the acetylation of natural dihydroquercetin-3-acetate (**7b**). When the reaction time was shortened from several hours to a few minutes, the pentaacetate (**4**) was replaced by dhq 3',4',7-triacetate (**2**) as major co-product of **3**, while the addition of a limited amount of acetic anhydride (three equivalents) to a solution of **1** and KOAc in aqueous MeOH[24] led to the formation of a complex mixture (*Scheme 2*) containing approximately equimolar amounts (by ^{1}H NMR) of **2**, dhq 3'4'-diacetate (**5a**), 3',7-diacetate (**5b**), 4',7-diacetate (**5c**), 3'-acetate (**5d**) and 4'-acetate (**5e**) as well as some 7-acetate (**6a**). These observations establish for the hydroxyl groups the relative reactivity order 3'/4'>7>3>5 which is almost identical



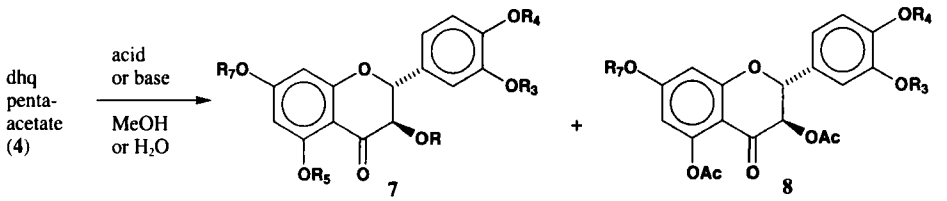
5
a) $R_3 = R_4 = Ac$, $R_7 = H$
b) $R_3 = R_7 = Ac$, $R_4 = H$
c) $R_4 = R_7 = Ac$, $R_3 = H$
d) $R_3 = Ac$, $R_4 = R_7 = H$
e) $R_4 = Ac$, $R_3 = R_7 = H$
+**2**) $R_3 = R_4 = R_7 = Ac$

6
a) $R = R_5 = H$
b) $R = H$, $R_5 = Ac$
c) $R = Ac$, $R_5 = H$
+**5a**

Scheme 2

(except 3'-OH) to the order of replacing acetyl by alkyl groups in quercetin derivatives.[25] Assuming deprotonation as the first step of the acylation mechanism, the high reactivity of the 3'-, 4'- and 7-OH groups is attributable to their relatively high acidities,[8] and the low reactivity of the 5-OH group to strong intramolecular hydrogen bonding via a planar hexagonal ring structure which lowers its acidity and imparts partial quinoid character to the A-ring.[20] It also implies that hydrogen bonding between the 3-OH and the carbonyl group and between the 3'- and 4'-OH groups is relatively weak and easily disrupted by polar solvent molecules. The formation of **6a** as major acetylation product, together with small quantities of 3,7-diacetate (**6c**), 5,7-diacetate (**6b**) and 3',4'-diacetate (**5a**), when the two adjacent B-ring hydroxyl groups and/or the 5-OH group are temporarily protected by complexation with borate[26] (*Scheme 2*) supports this interpretation.

Hydrolysis with dilute aqueous sulfuric acid converts dhq pentaacetate (**4**) mainly to 3,3',4',5-tetraacetate (**8a**) (the chief contaminant of samples purified by PTLC or by crystallization from MeOH) and 3,3',4'-triacetate (**7a**), as well as smaller amounts of 3,4',5-triacetate (**8b**), 3,3',5-triacetate (**8c**), 3,5,7-triacetate (**8d**), 3,5-diacetate (**8g**) and 5-acetate (**7e**), and traces of 3,4',5,7-tetra-acetate (**8e**) and 3,3',5,7-tetraacetate (**8f**) (*Scheme 3*).



a) $R_5 = R_7 = H$, $R = R_3 = R_4 = Ac$
b) $R_3 = R_4 = R_5 = R_7 = H$, $R = Ac$
c) $R = R_4 = Ac$, $R_3 = R_5 = R_7 = H$
d) $R = R_3 = Ac$, $R_4 = R_5 = R_7 = H$
e) $R = R_3 = R_4 = R_7 = H$, $R_5 = Ac$

a) $R_7 = H$, $R_3 = R_4 = Ac$
b) $R_3 = R_7 = H$, $R_4 = Ac$
c) $R_4 = R_7 = H$, $R_3 = Ac$
d) $R_3 = R_4 = H$, $R_7 = Ac$
e) $R_3 = H$, $R_4 = R_7 = Ac$
f) $R_4 = H$, $R_3 = R_7 = Ac$
g) $R_3 = R_4 = R_7 = H$

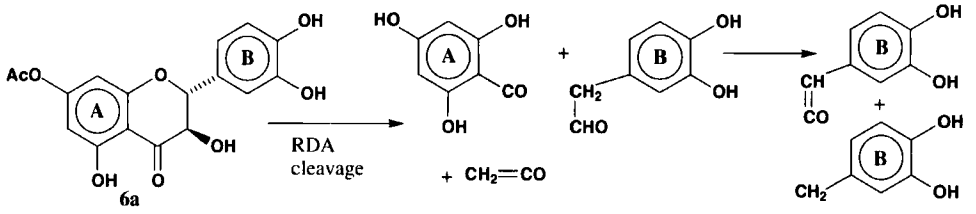
Scheme 3

The ^1H NMR signal at δ 2.36 (in $CDCl_3$) typical of a C-5 acetoxy group,[27] the absence of a chelated phenolic OH singlet at δ 11.3 and the chemical shifts of the C-ring protons (see below) clearly distinguish **8a** from **3**, proving that the products of dihydroquercetin acetylation and pentaacetate deacetylation are not identical as previously claimed,[11] but regioisomeric. Hydrolysis and methanolysis in the presence of trifluoroacetic acid, formic acid, acetic acid or potassium acetate as catalysts lead to the same products although the relative mole percentages vary with reaction time and temperature. Even solid crude penta-acetate was found to hydrolyze gradually to **8a** on prolonged exposure (several months) to humid air. The 3,3',4',7-tetraacetate (**3**) is converted mainly to the triacetate **7a** under these conditions. Thus the sterically most accessible 7-acetoxy group undergoes ester cleavage most rapidly, the alkyl ester (3-OAc) resists cleavage, and the approximate relative reactivity sequence of the five acetoxy groups of **4** is 7>5>3'/4'>3. The structures and relative amounts of the various acetates formed in the acid-catalyzed deacetylation of **4** reflect this reactivity order: Solvolysis of the 5-acetoxy or of a B-ring acetoxy group of **8a**, the main initial product, leads to the formation of the triacetates **7a**, **8b** and

8c which are subsequently converted to the 3- and 5-monoacetates (**7b** and **7e**) *via* the diacetates **7c**, **7d** and **8g**. The high reactivity of the 7-acetoxy group prevents the accumulation of significant quantities of those tetra- and triacetates (**8d**, **8e** and **8f**) that are fully acetylated in the A-ring.

The reaction of **3** or **4** with aqueous methanol (40-44 hours/25°) in the presence of a weak base catalyst such as sodium sulfite,[28] imidazole or acetate results in complete solvolysis of all aryl ester linkages and predominant formation of dihydroquercetin 3-acetate (**7b**), accompanied only by trace quantities of the 3,3',4'-triacetate (**7a**), 3,3'-diacetate (**7d**) and 3,4'-diacetate (**7c**). The peracetylation of (+)-dihydroquercetin, followed by base-catalyzed regiospecific solvolysis of **4**, therefore constitutes a convenient, economic synthetic route to **7b**, the sweet dihydroflavonol previously extracted from several plants.[3,15,22,29] Its racemic form was first prepared by Freudenberg and Weinges[30] by hydrogenolysis of its tetrabenzyl ether. Except for the ^{1}H NMR chemical shifts in deuterated dimethylsulfoxide,[31] our spectral data for **7b** are in complete agreement with those reported in the literature.[4,15,22]

The mass spectra of the dihydroquercetin acetates show weak molecular ion peaks (M^+) and very similar fragmentation patterns.[4,22] The most characteristic fragmentation modes are rapid, successive loss of ketene units (M^+-n•42, where n = 1 to 5) and/or acetic acid (M^+-60), and retro-Diels-Alder (RDA) cleavage. This latter process generates a deacetylated, protonated A-ring fragment (m/z 153) as the signal of highest intensity and a strong B-ring fragment at m/z 152. Other prominent peaks appear at m/z 286 (loss of water), 150 and 123, as observed for dihydroquercetin itself.[32] The latter two are ascribed to 3,4-dihydroxyphenylketene and 3,4-dihydroxybenzyl cations, respectively, and formed by secondary cleavage and rearrangement of the RDA fragment with the B-ring (Scheme 4). This interpretation was confirmed by recording a high-resolution mass spectrum of **6a** as representative sample.



Scheme 4

In the ^{1}H NMR spectra (Table 1), acetylation of the 3'- and/or 4'-OH group is found to induce the expected downfield shifts in the aromatic B-ring signals: by 0.19-0.32 ppm for the ortho-, 0.13-0.24 ppm for the meta- and 0.40-0.50 ppm for the para-proton; the A- and C-ring substitution patterns (-OH vs -OAc at C-3/5/7) have little, if any, influence on the chemical shifts of H-2'/5'/6' (Table 2). Assignment of the aromatic A-ring protons (H-6 vs. H-8), which are also deshielded by O-acetylation (by 0.30-0.40 ppm per acetyl), is more difficult but fortunately unnecessary for structure identification. The NOE produced by saturation of the acetoxy protons is weak[17] and peak overlap in the δ 2.25-2.30 region complicates deconvolution. However, the distinction between 7- and 5-acetoxy isomers is facilitated by the appearance of a sharp singlet at δ 11-12 for the

chelated 5-OH proton of the former and by the consistently larger difference between the H-6 and H-8 chemical shifts of the latter.

TABLE 1. Proton Chemical Shifts of *trans*-Dihydroquercetin (dhq) Acetates

Cmpd: Position of O-acetylation	H-2	H-3	H-6/8	H-2'	H-5'	H-6'	5-OR[a]	7-OR[a]	3',4'-OR[a]	3-OR[a]
dhq (**1**)	5.01	4.60	5.98/5.93	7.06	6.85	6.90	11.71	9.7	8.0	4.76
3-OAc (**7b**)	5.35	5.82	6.00/5.98	7.05	6.86	6.89	11.57	9.8	8.1	1.96
3'-OAc (**5d**)	5.10	4.62	5.99/5.95	7.27	7.00	7.31	11.69	9.8	8.7/2.26	4.85
4'-OAc (**5e**)	5.11	4.63	6.00/5.97	7.19	7.09	7.09	11.69	9.8	8.7/2.26	4.79
5-OAc (**7e**)	4.95	4.44	6.33/6.28	7.07	6.85	6.92	2.27	10.0	8.1	4.30
7-OAc (**6a**)	5.15	4.76	6.31/6.28	7.08	6.86	6.93	11.60	2.25	8.1	4.88
3,5-$(OAc)_2$ (**8g**)	5.31	5.65	6.36/6.29	7.05	6.86	6.89	2.25	9.8	8.3	1.93
3,7-$(OAc)_2$ (**6c**)	5.49	5.97	6.36/6.33	7.07	6.87	6.88	11.40	2.26	8.1	1.98
3,3'-$(OAc)_2$ (**7d**)	5.43	5.83	6.02/6.01	7.24	7.02	7.31	11.54	9.9	8.8/2.26	1.98
3,4'-$(OAc)_2$ (**7c**)	5.47	5.84	6.02/6.00	7.18	7.11	7.05	11.56	9.9	8.8/2.26	1.97
3',7-$(OAc)_2$ (**5b**)	5.23	4.78	6.34/6.32	7.30	7.01	7.33	11.59	2.25	8.7/2.26	5.02
4',7-$(OAc)_2$ (**5c**)	5.25	4.79	6.33/6.30	7.21	7.09	7.09	11.59	2.25	8.7/2.26	5.06
3',4'-$(OAc)_2$ (**5a**)	5.22	4.65	6.01/5.99	7.48	7.31	7.52	11.69	9.9	2.28	4.96
5,7-$(OAc)_2$ (**6b**)	5.11	4.61	6.75/6.62	7.10	6.86	6.94	2.30	2.27	8.1	4.47
3,3',4'-$(OAc)_3$ (**7a**)	5.57	5.85	6.03	7.45	7.34	7.53	11.53	9.9	2.28	1.99
3',4',7-$(OAc)_3$ (**2**)	5.36	4.81	6.35/6.34	7.50	7.32	7.54	11.57	2.25	2.28	5.17
3,3',5-$(OAc)_3$ (**8c**)	5.39	5.66	6.37/6.30	7.25	7.02	7.33	2.25	10.0	8.7/2.26	1.93
3,4',5-$(OAc)_3$ (**8b**)	5.42	5.67	6.38/6.31	7.19	7.10	7.06	2.25	10.0	8.7/2.26	1.94
3,5,7-$(OAc)_3$ (**8d**)	5.45	5.79	6.80/6.65	7.08	6.87	6.92	2.28	2.28	8.1	1.94
3,3',5,7-$(OAc)_4$ (**8f**)	5.55	5.82	6.82/6.67	7.29	7.03	7.37	2.28	2.28	2.26	1.96
3,4',5,7-$(OAc)_4$ (**8e**)	5.57	5.83	6.83/6.68	7.23	7.12	7.09	2.28	2.28	2.26	1.96
3,3',4',5-$(OAc)_4$ (**8a**)	5.53	5.68	6.41/6.32	7.47	7.34	7.55	2.26	9.9	2.28	1.95
3,3',4',7-$(OAc)_4$ (**3**)	5.71	6.00	6.39	7.48	7.35	7.56	11.35	2.26	2.28	2.00
dhq$(OAc)_5$ (**4**)	5.69	5.84	6.86/6.69	7.50	7.35	7.59	2.28	2.28	2.28	1.97

Solvent: acetone-d_6 (δ 2.04 ppm); a) R = H or Ac; J_{23} = 11.4-12.3 Hz, J_{68} = 2.0-2.4 Hz, $J_{2'6'}$ = 1.8-2.2 Hz and $J_{5'6'}$ = 8.1-8.4 Hz

TABLE 2. Chemical Shift Ranges (ppm) for B-ring Protons

Substituents	Cmpds	H-2'	H-5'	H-6'
3',4'-$(OH)_2$	**1, 6a, 6b, 6c, 7b, 8d, 8g, 7e**	7.05-7.10	6.85-6.87	6.89-6.94
3'-OH-4'-OAc	**5c, 5e, 7c, 8b, 8e**	7.18-7.23	7.09-7.12	7.05-7.09
3'-OAc-4'-OH	**5b, 5d, 7d, 8c, 8f**	7.24-7.30	7.00-7.03	7.31-7.33
3',4'-$(OAc)_2$	**2, 3, 4, 5a, 7a, 8a**	7.45-7.50	7.31-7.35	7.52-7.56

The C-ring protons (*trans*-H-2/3) serve as sensitive probes for the number and positions of acetoxy groups in the rest of the molecule (Table 3). Since hydrogen bonding reduces the electron density at these sites, 5-O-acetylation induces an upfield shift whereas 7-O-acetylation and esterification of the B-ring (3'- and/or 4'-OH) deshield H-2 and H-3. The effects of acetylation on the magnetic environment of H-3 are additive, permitting calculation of its chemical shift, e.g., for dhq pentaacetate: δ (H-3) = 4.60 (dihydroquercetin) + 1.20 + 0.02 + 0.02 - 0.16 + 0.15 = 5.83 (found: 5.84). In $CDCl_3$, the shift increments were found to be considerably smaller than in acetone-d_6: +0.60 ppm for 3-, -0.12 ppm for 5- and +0.08 ppm for 7-O-acetylation of **2**, **7a** and **8a**, respectively. In isomers differing only in the position of B-ring acetylation, the heterocyclic ring protons of the 4'-acetate were always found slightly downfield from those of the 3'-acetate.

TABLE 3. H-2/3 Acetylation Shift Increments (ppm)[a]

Acetylation of	3-OH	3'-OH	4'-OH	5-OH	7-OH
Shift increment for H-2	+0.35	+0.10	+0.12	-0.04	+0.15
Shift increment for H-3	+1.20	+0.02	+0.02	-0.16	+0.15

a) + = downfield, - = upfield

In summary, we have shown that several partial dihydroquercetin acetates are accessible on a preparative scale: the 3,3',4',7-tetraacetate (**3**) and the 3',4',7-triacetate (**2**) by slow and rapid base-catalyzed acetylation of dihydroquercetin, respectively, the 3,3',4',5-tetraacetate (**8a**) and the 3,3',4'-triacetate (**7a**) by acid- or base-catalyzed deacetylation of the pentaacetate (**4**) and **3**, respectively, the 3-acetate (**7b**) by base-catalyzed deacetylation of **4** or **3**, and the 7-acetate (**6a**) by borate-catalyzed acetylation. Eight new regioisomers of these six mono-, tri- and tetraacetates as well as eight new diacetates were formed as minor products and isolated by PTLC or flash chromatography. Analysis of their proton NMR spectra reveals regularities that permit an estimation of the chemical shifts of the remaining eight unknown structures and will assist in the optimization of the reaction conditions for their preparation, a project currently in progress. Selectively alkylated dihydroflavonols will then be synthesized by methylation/deprotection of the appropriate acetates and tested for their biological activities.

EXPERIMENTAL SECTION

Acetic anhydride (Ac_2O) and pyridine were purchased from Sigma-Aldrich and used without further purification, dihydroquercetin (dhq) was extracted from Douglas fir bark.[5,9] Melting points (uncorrected) were recorded on a Fisher-Johns melting point apparatus, optical rotations on a Perkin-Elmer 241 polarimeter, and NMR spectra on a Bruker spectrometer (400 MHz). Chemical shifts are reported relative to the acetone-d_6 peaks centered at δ 2.04 (^{1}H NMR) and 29.80 ppm (^{13}C NMR), the DMSO-d_5 quintet at 2.50 ppm or the $CHCl_3$ proton resonance at 7.26 ppm. Evaporations were carried out on a water bath (70-80°) or at reduced pressure on a rotary evaporator. Analytical thin layer chromatography (TLC) was performed on Merck Kieselgel 60F-254 (0.2 mm) DC-Plastikfolien, and preparative thin layer chromatography (PTLC) on Merck Kieselgel 60HF-254/366 (0.75 mm) with

benzene/acetone (4:1 v/v) as eluent. Elemental analyses were run on a Carlo-Erba elemental analyzer model 1106, low-resolution mass spectra on an HP-5985 quadrupole instrument, high resolution mass spectra on a Kratos Concept 1H double focussing instrument (70 eV); exact mass measurements (by peak matching in triplicate, by full scan at 10 sec/decade) were carried out at 10,000 R.P. using pfk as internal standard, and peak intensities are reported relative to the protonated RDA fragment of dihydroquercetin at m/z 153 which was the most intense peak above m/z 100 in all cases.

Dihydroquercetin Pentaacetate (4) was prepared according to literature procedures[12,14] from dhq, acetic anhydride and sodium acetate or excess pyridine as base catalyst. ^{1}H NMR spectroscopy showed the crude product to be contaminated with other dhq acetates. Purification by PTLC (R_f 0.68) and recrystallization (EtOH) afforded a white solid melting at 144-146°, lit.[14] 147-148° (see also discussion section).

Anal. Calcd. for $C_{25}H_{22}O_{12}$: C, 58.37; H, 4.31. Found: C, 58.21; H, 4.24

^{13}C NMR (Me_2CO-d_6): δ (ppm) 186.0 (C-4), 169.3/169.1/168.7/168.6/168.5 (OAc), 163.4 (C-8a), 157.7/152.5 (C-5/7), 144.3/143.5 (C-3'/4'), 135.1 (C-1'), 126.4 (C-6'), 124.7/124.1 (C-2'/5'), 112.4/109.8 (C-6/8), 111.7 (C-4a), 81.2 (C-2), 74.1 (C-3) and 21.0/20.9/20.5/20.2 (OAc). In the ^{1}H NMR spectrum[14,16,17] ($CDCl_3$), a NOESY experiment identified the δ 6.60 signal as H-6 (enhanced by both the 5-OAc at δ 2.37 and the 7-OAc protons at δ 2.30) and the δ 6.78 signal as H-8 (enhanced only by 7-OAc); in acetone-d_6 the H-6' signal (δ 7.59) appeared as a ddd, due to ortho- (8.4 Hz), meta- (2.1 Hz) and benzylic coupling (0.4 Hz), which collapsed to a dd on decoupling H-2 at δ 5.69; NOE of both H-2 and H-3 on saturation of either H-2' or H-6' demonstrates free rotation of the B-ring.

Dihydroquercetin 3,3',4',7-Tetraacetate (3) and 3',4',7-Triacetate (2).- One drop of pyridine was added to a suspension of 305 mg dhq (1.00 mmol) in 1.0 mL acetic anhydride (10 mmol), affecting complete dissolution after 2 min of stirring at 25°. TLC monitoring revealed the initial presence of **2** (R_f 0.52) and its rapid disappearance (within 30 min) in favor of **3** (R_f 0.74) and **4** (R_f 0.70). After 90 min the pasty mixture was vigorously shaken with 10 mL water (which resulted in solidification of the oily product), washed by decantation (8 mL water), dissolved in 15 mL hot MeOH and allowed to crystallize at 25°, giving 240 mg of **3**, mp. 144-146° (lit.[11,12] 151-154°). The gummy evaporation residue (180 mg) of the filtrate was found (by ^{1}H NMR) to contain **3** and **4** in the molar ratio 2:1, for a total yield of 75% **3** and 12% **4**. The structure of the tetraacetate (**3**) was verified by ^{1}H NMR in acetone-d_6 (Table 1) and in $CDCl_3$:[22] δ (ppm) 11.28 (s, 5-OH), 7.39 (dd, J = 8.5/2.1 Hz, H-6'), 7.30 (d, J = 2.1 Hz, H-2'), 7.27 (d, J = 8.5 Hz, H-5'), 6.39/6.33 (2d, J = 2.1 Hz, H-6/8), 5.74 (d, J = 12.0 Hz, H-3), 5.41 (d, J = 12.0 Hz, H-2), 2.31/2.30 (9H, ArOAc) and 2.09 (3-OAc).

Anal. Calcd. for $C_{23}H_{20}O_{11}$: C, 58.48; H, 4.27. Found: C, 58.30; H, 4.27

Application of the same procedure, except for quenching in water after only 2 min reaction time, afforded the triacetate (**2**) in 26 % yield, together with 54% **3** from which it was separated by PTLC (R_f 0.54 vs. 0.72): white feathers (EtOH), mp. 77-79°, ^{1}H NMR (Table 1); HREIMS *m/z* 430.0898 (calcd. 430.0899 for $C_{21}H_{18}O_{10}$). With KOAc (instead of pyridine) as base catalyst, **2** was the major (56% yield) and **3** the minor product (28%).

Acetylation of Dihydroquercetin (dhq) in Aqueous Methanol.- Acetic anhydride (155 µL, 1.60 mmol) was added to a solution of 152 mg dhq (0.50 mmol) and 296 mg KOAc (3.00 mmol) in 3 mL 50% aq. MeOH. After 30 seconds of stirring, the reaction mixture was acidified with 10 mL 0.2 M H_2SO_4 and extracted with EtOAc. The yellow organic layer was washed until acid-free, dried ($MgSO_4$) and evaporated to give 162 mg of a light brown resin which was found (by ^1H NMR) to contain approximately equimolar quantities of **2**, dhq 3',4'-diacetate (**5a**), 3',7-diacetate (**5b**), 4',7-diacetate (**5c**), 3'-acetate (**5a**) and 4'-acetate (**5e**) as well as traces of 7-acetate (**6a**) and unreacted dhq. Small samples of the individual components were obtained by PTLC and analyzed by ^1H NMR (Table 1) and mass spectrometry. **2** (R_f 0.54): see above. **5a** (R_f 0.45): beige crystals, mp. 93-95° (from EtOH), HREIMS *m/z* 388.0792 (calcd 388.0794 for $C_{19}H_{16}O_9$). **5b** and **5c** (1:1, R_f 0.40): HREIMS *m/z* 388.0794 (calcd 388.0794 for $C_{19}H_{16}O_9$). **5d** and **5e** (1:1, R_f 0.32): HREIMS *m/z* 346.0690 (calcd 346.0688 for $C_{17}H_{14}O_8$). The two monoacetates (**5d** and **5e**) were formed as major products (50% yield), together with smaller quantities of diacetates (16% **5a**, 5% **5b**, 5% **5c**) and the tri-acetate **2** (2%), for a total yield of 78%, when the reaction was performed with 3 equivalents of Na_2SO_3 as base catalyst (1h stirring).

Borate-catalyzed Acetylation of Dihydroquercetin.- To an ice-cooled solution of 152 mg (0.50 mmol) dhq in 1 mL MeOH were added 1.0 mL 3M aq. sodium borate and 155 mL (1.6 mmol) acetic anhydride. After 2 min stirring the reaction was quenched with 10 mL 0.2M H_2SO_4 and extracted with EtOAc (3x5 mL). The combined organic layers were washed (3x5 mL water), dried ($MgSO_4$) and evaporated to give a yellow-brown resinous product mixture (173 mg) which was estimated (by ^1H NMR) to contain **1** (R_f 0.17), **2** (R_f 0.54), dhq 7-acetate (**6a**) and dhq 5,7-diacetate (**6b**) in the molar ratio 1:1.3:3.6:0.8 and separated by PTLC. **6a** (R_f 0.28): HREIMS (see Scheme 4) *m/z* (%) 346.0695 (40, M^+) [calcd 346.0688 for $C_{17}H_{14}O_8$], 317.0669 (27, M-CHO), 275.0558 (34, dhq-CHO), 195.0287 (12, RDA fragment A+1) [calcd 195.0293 for $C_9H_7O_5$], 165.0189 (10, RDA fragment A-CHO) [calcd 165.0188 for $C_8H_5O_4$], 153.0192 (100, RDA fragment A of dhq+1) [calcd 153.0187 for $C_7H_5O_4$], 152.0476 (31, RDA fragment B of dhq) [calcd 152.0473 for $C_8H_9O_3$], 152.0110 (8, RDA fragment A of dhq) [calcd 152.0109 for $C_7H_4O_4$], 150.0336 (47, RDA fragment B-2) [calcd 150.0316 for $C_8H_6O_3$], 123.0447 (42, RDA fragment B of dhq-CHO) [calcd 123.0446 for $C_7H_7O_2$]. **6b** (R_f 0.30) HREIMS: *m/z* 388.0789 (calcd 388.0794). Dhq 3,7-diacetate (**6c**) and 3',4'-diacetate (**5a**) were identified as main components of a weak band at R_f 0.43.

Dihydroquercetin 3,3',4',5-Tetraacetate (8a), 3,3',4'-Triacetate (7a) and 3,5-Diacetate (8g).- A solution of 29.7 mg KOAc (0.30 mmol) in 1.0 mL MeOH was added dropwise to a suspension of 52.2 mg (0.10 mmol) of **4** in 1.0 mL MeOH. The starting material gradually dissolved and the solution turned orange during 18 h of stirring at 25°. Acidification (0.5 mL 1M HCl), dilution with water, extraction (EtOAc), washing (H_2O), drying ($MgSO_4$) and solvent evaporation gave 45.0 mg of a light brown resin containing **8a** (53% yield), 3,5-diacetate (**8g**) (20%) and 3,3',4'-triacetate (**7a**) (10%). The products were separated by PTLC and analyzed by ^1H NMR (Table 1) and MS. **8a** (R_f 0.58): HREIMS *m/z* 472.1005 (calcd 472.1005 for $C_{23}H_{20}O_{11}$); **8g** (R_f 0.38): HREIMS *m/z* 388.0796 (calcd 388.0794 for $C_{19}H_{16}O_9$); and **7a** (R_f 0.61): HREIMS *m/z* 430.0892 (calcd 430.0899 for $C_{21}H_{18}O_{10}$).

The triacetate (**7a**) was formed as major product (48% yield) on refluxing a methanolic solution of **3** for 24 h; separation from unreacted **3** (R_f 0.74) by PTLC.

Acid-catalyzed Partial Deacetylation of Dihydroquercetin Pentaacetate (4).- A suspension of 103 mg **4** (0.20 mmol) in 2.0 mL 1.0 M aq. H_2SO_4 was stirred for 2 h at 40°. Extraction (3x3 mL EtOAc), washing (4 mL aq. $NaHCO_3$, 2x4 mL water), drying ($MgSO_4$) and solvent evaporation (70°) afforded 88 mg of a yellow oil which was found (by ^1H NMR) to contain **8a** (22% yield), **7a** (18%) and **8g** (9%) as well as several new partial dhq acetates. The following compounds were separated by PTLC and identified by ^1H NMR (Table 1) and MS. Dhq 5-acetate (**7e**, R_f 0.21, with dhq, 4.3 mg): HREIMS *m/z* 346.0693 (calcd 346.0688 for $C_{17}H_{14}O_8$); dhq 3,4',5- , 3,3',5- and 3,5,7-triacetate (**8b/8c/8d** 2:2:1, R_f 0.49, 19.8 mg): HREIMS *m/z* 430.0912 (calcd 430.0899 for $C_{21}H_{18}O_{10}$); dhq 3,4'- and 3,3'-diacetate (**7c/7d** 1:1, R_f 0.54, 5.1 mg): HREIMS *m/z* 388.0792 (calcd 388.0794 for $C_{19}H_{16}O_9$); and dhq 3,4',5,7- and 3,3',5,7-tetraacetate (**8e/8f**, R_f 0.59 with **8a**).

Dihydroquercetin 3-Acetate (7b).- A solution of 51.5 mg (0.10 mmol) of **4** in 1.6 mL MeOH was added to a stirred solution of 37.6 mg Na_2SO_3 (0.30 mmol) in 1.6 mL water. The initially formed yellow precipitate dissolved after 3 h. Stirring was continued for another 41 h. Acidification with dilute HCl, extraction with EtOAc (4x1 mL), washing (H_2O, aq. $NaHCO_3$), drying ($MgSO_4$) and solvent evaporation gave 24.1 mg (70%) crude **7b** which was purified by PTLC and recrystallization (aq. MeOH); pale yellow needles, mp. 125-127° (lit.[30] 126-128°); ^1H NMR (Me_2CO-d_6):[22] Table 1; ^1H NMR (DMSO-d_6):[32] δ (ppm) 11.45 (OH-5), 9.1 (OH-3',4',7), 6.89 (br s, H-2'), 6.74 (br s, H-5'/6'), 5.94/5.91 (2d, J = 2.0 Hz, H-6/8), 5.82 (d, J = 11.8 Hz, H-3), 5.40 (d, J = 11.8 Hz, H-2) and 1.96 (s, OAc); ^{13}C NMR (Me_2CO-d_6): δ 193.0 (C-4), 169.5 (3-OAc), 168.0 (C-7), 165.1 (C-5), 163.8 (C-8a), 147.0/145.9 (C-3'/4'), 128.2 (C-1'), 120.6 (C-6'), 115.9/115.4 (C-2'/5'), 102.0 (C-4a), 97.4/96.3 (C-6/8), 81.9 (C-2), 73.0 (C-3) and 20.2 (3-OAc).

Stirring a suspension of 0.10 mmol **4** in 1.0 mL 0.50 M aq. Na_2SO_3 for 24 h, acidification and workup as above afforded a mixture (34.6 mg) containing **7b**, **4**, **8a**, **7c**, **7d** and **7a** in the approximate molar ratio 50:20:10:8:8:4 (by ^1H NMR analysis).

Acknowledgements.- I thank D. McGillivray (University of Victoria, B.C.) for recording the mass spectra and L. Andronic for technical assistance.

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† Presented in part at the 81. Canadian Society for Chemistry Conference, Whistler, B.C., Canada, 2 June 1998

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(Received May 12, 1998; in final form July 7, 1998)