Methylation of Dihydroquercetin Acetates: Synthesis of 5-*O*-Methyldihydroquercetin

Eberhard Kiehlmann*,† and Peter W. Slade‡

Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Received August 14, 2003

The major products of methylation of dihydroquercetin (**1**, dhq) with diazomethane have been identified as 7-*O*-methyldhq (**9**), 7,3′-di-*O*-methyldhq (**10**), 7,4′-di-*O*-methyldhq (**11**), and 7,3′,4′-tri-*O*-methyldhq (**2**). With dhq 3,7,3′,4′-tetraacetate (**6**), dhq 3,5,3′,4′-tetraacetate (**5**), dhq 3,3′,4′-triacetate (**7**), dhq 7,3′,4′-triacetate (**8**), and dhq 3-acetate (**4**) the same reaction affords mainly 5-*O*-methyldhq 3,7,3′,4′-tetraacetate (**15**), 7-*O*-methyldhq 3,5,3′,4′-tetraacetate (**13**), 7-*O*-methyldhq 3,3′,4′-triacetate (**14**), 5-*O*-methyldhq 7,3′,4′-triacetate (**25**), and 7-*O*-methyldhq 3-acetate (**12**), respectively. The methylation of **8** is accompanied by intermolecular acetyl migration from phenolic oxygen to the hydroxy group of the heterocyclic ring. 5-*O*-Methyldhq (**28**) is accessible by deacetylation of **25**. 5,7-Di-*O*-methyldhq (**29**), four known methyl ethers, 13 new and three known methyl ether acetates of dhq, and six new isomers with 2,3-*cis* stereochemistry were spectroscopically identified.

Dihydroflavonols are found in many plants and have been shown to be intermediates in the biosynthesis of other flavonoid classes. The most common member of this family is dihydroquercetin (**1**, dhq),[1] which occurs in nature as free phenol, as glycoside, and in the form of free and glycosylated phenol ethers or esters. Seven optically active methyl ethers and four methyl ether 3-acetates of dhq have been isolated from plants:[2] dhqMe5,[3,4] dhqMe7 (padmatin), dhqMe3′,[5,6] dhqMe4′,[7−9] dhqMe73′, dhqMe74′,[9] *cis*-dhqMe74′,[9] dhqMe7Ac3, dhqMe3′Ac3,[10] dhqMe4′Ac3,[11] and dhqMe73′Ac3.[12] All except one (*cis*-dhqMe74′) have the 2,3-*trans* configuration, and only one of them (dhqMe5) has a 5-*O*-methyl group. Neoastilbin, a dhq 3-*O*-rhamnoside,[13] and dhqAc3 are responsible for the sweetness of the herb *Tessaria dodoneifolia*; dhqMe4′, dhqAc3, and dhqMe4′Ac3, respectively, were rated by Kinghorn et al.[14] to be 40, 80, and 400 times sweeter than sucrose. Many flavonoids of similar structures are known to be effective radical scavengers (antioxidants).[15]

The structures of the natural products have been determined by NMR and UV spectroscopy and were not verified by independent synthesis because efficient procedures for the regioselective alkylation of polyhydroxy-dihydroflavonols at only one or two phenolic oxygens are not available. Alkyl halides and dimethyl sulfate are known to convert dhq mainly to the tri- and tetra-*O*-methyl ethers dhqMe73′4′ (**2**) and dhqMe573′4′ (**3**) in alkaline solution, and several methylated oxidation and rearrangement byproducts (quercetins, alphitonins, aurones, and chalcones) result from attack of the labile pyranone ring by base.[16] With excess diazomethane in methanol, these side reactions are not observed, but **2** and **3** are formed in much lower yields.[16,17] We confirmed these findings and reasoned that our recently described dhq tri- and tetraacetates[18] should permit access to specific di- and monomethyl ethers by reaction of their free phenolic OH groups with CH_2N_2 and subsequent deprotection. The results of our experiments with dhq (**1**), dhqAc3 (**4**), dhqAc353′4′ (**5**), dhqAc373′4′ (**6**), dhqAc33′4′ (**7**), and dhqAc73′4′ (**8**) are reported in this paper.

Results and Discussion

Of the four phenolic hydroxy groups of dhq, 7-OH is most and 5-OH least acidic.[19] Their reactivities toward CH_2N_2 decrease in the same order. Thus from dhq we obtained mainly dhqMe7 (**9**), exclusively in the presence of sodium borate, and smaller amounts of dhqMe73′ (**10**), dhqMe74′ (**11**), and dhqMe73′4′ (**2**) (Scheme 1a), while only dhqMe7Ac3 (**12**) was formed from dhqAc3 (**4**). The ^{1}H NMR data of our synthetic samples (Table 1) agree with those listed in the literature and thereby confirm the structures initially assigned to the natural products **9**,[20] **10**[21], **11**,[7,9] and **12**.[20] However, our proton chemical shifts of *cis*-dhqMe74′ (**11a**, see Table 1), which was recently claimed to co-occur with the *trans*-diastereomer in the stem bark of *Lannea coromandelia*, differ from the reported values.[9] We identified **11a** as one of the four di-*O*-methyldihydroquercetins obtained by methylation of an equimolar mixture of *trans*- and *cis*-dhq[22] and chromatographic separation from the mono- and trimethylated coproducts. Although the four isomers (*trans*-dhqMe73′, *trans*-dhqMe74′, *cis*-dhqMe73′, and *cis*-dhqMe74′) were not separable from each other, knowledge of the NMR spectra of the two 2,3-*trans* structures permitted peak assignments for their respective *cis*-diastereomers (Table 1). The *trans*-isomer predominated in each of the PTLC fractions, indicating slow *cis* → *trans* isomerization during and after the reaction.

The ^{1}H NMR spectra reveal several interesting features of diagnostic value for the distinction between *cis*- and *trans*-dihydroflavonols. The C-ring proton chemical shifts of the five diastereomeric pairs listed at the top of Table 1 (**1**, **2**, **9**, **10**, **11** and their *cis* isomers **1a**, **2a**, **9a**, **10a**, **11a**) are significantly affected by the 2,3-stereochemistry but vary only slightly with the number of methoxy groups in rings A and B. In each case, changing the configuration from *trans* to *cis* shifts the H-2 resonance downfield by 0.40 ppm and H-3 upfield by 0.34−0.41 ppm; that is, *cis*-structures differ from their *trans*-diastereomers not only by their much smaller J_{23} coupling constant (2−3 Hz vs 11−12 Hz) but also by a larger H-2/H-3 peak separation.

* To whom correspondence should be addressed. Tel: 604-291-4880. Fax: 604-291-3765. E-mail: ekie@sfu.ca.
† Simon Fraser University.
‡ Present address: University College of the Fraser Valley, 33844 King Rd., Abbotsford, B.C. V2S 7M8.

10.1021/np034005w CCC: $25.00
Published on Web 11/26/2003

Scheme 1. Methylation of dhq (**1**), dhqAc353′4′ (**5**), dhqAc373′4′ (**6**), and dhqAc33′4′ (**7**)

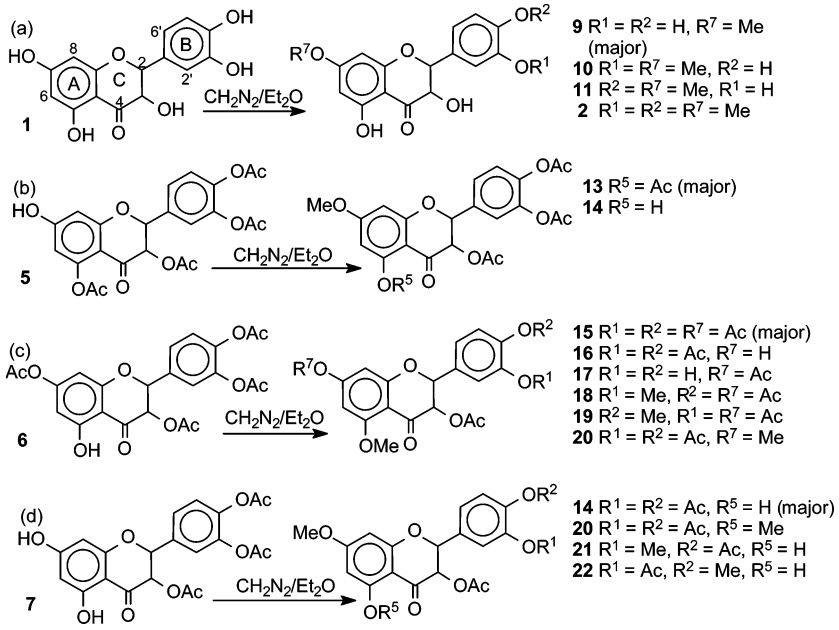


Table 1. Proton Chemical Shifts of Dihydroquercetin Derivatives[a]

compound	#	H-2	H-3	J_{23}	H-6/8	H-2′	H-5′	H-6′	5-OR	7-OR	3′,4′-OR	3-OR
dhq	**1**	5.01	4.60	11.4	5.98/5.94	7.06	6.85	6.90	11.71	9.69	7.98/8.04	4.69
cis-dhq	**1a**	5.41	4.26	2.7	5.99/5.95	7.09	6.80	6.89	11.85			5.14
dhqMe73′4′	**2**	5.13	4.73	11.4	6.08/6.05	7.22	6.99	7.11	11.69	3.85	3.83	4.78
cis-dhqMe73′4′	**2a**	5.53	4.32	2.5	6.12/6.06	7.22	6.94	7.11	11.81	3.81	3.80	5.27
dhqMe573′4′	**3**	4.98	4.46	12.1	6.23/6.13	7.21	6.98	7.10	3.86	3.86	3.83	4.27
cis-dhqAc33′4′	**7a**	5.88	5.70	2.6	6.04/6.11	7.43	7.28	7.43	11.71	9.98	2.27/2.28	1.93
dhqMe7	**9**	5.04	4.63	11.6	6.07/6.04	7.06	6.85	6.91	11.69	3.85	8.06	4.75
cis-dhqMe7	**9a**	5.44	4.28	2.6	6.09/6.04	7.09	6.81	6.89	11.81	3.85	8.06	5.20
dhqMe73′	**10**	5.10	4.72	11.3	6.08/6.05	7.21	6.87	7.03	11.69	3.87	3.85/7.79	4.77
cis-dhqMe73′	**10a**	5.49	4.31	2.6	6.11/6.04	7.21	6.82	7.03	11.82	3.84	3.84/7.70	5.25
dhqMe74′	**11**	5.08	4.66	11.3	6.08/6.05	7.00	6.97	7.09	11.68	3.87	7.73/3.85	4.76
cis-dhqMe74′	**11a**	5.49	4.28	2.6	6.11/6.04	7.00	6.93	7.09	11.80	3.83	7.65/3.83	5.22
cis-dhqMe74′ (ref 9)		6.10	5.02	3.2	6.14/6.00	6.84	6.89	6.77	11.65	3.88	7.71/3.80	
dhqMe7Ac3	**12**	5.38	5.84	11.8	6.10/6.08	7.06	6.85	6.88	11.53	3.87	8.4	1.95
dhqMe7Ac353′4′	**13**	5.57	5.71	12.3	6.56/6.42	7.48	7.34	7.56	2.26	3.91	2.28	1.95
dhqMe7Ac33′4′	**14**	5.60	5.87	11.9	6.13/6.15	7.44	7.34	7.53	11.49	3.88	2.28	1.99
cis-dhqMe7Ac33′4′	**14a**	5.91	5.72	2.6	6.14/6.22	7.43	7.28	7.43	11.65	3.90	2.27	1.93
dhqMe5Ac373′4′	**15**	5.59	5.68	12.1	6.56/6.47	7.47	7.33	7.54	3.86	2.26	2.28	1.98
dhqMe5Ac33′4′	**16**	5.46	5.55	11.9	6.20/6.09	7.43	7.32	7.50	3.81	9.72	2.27/2.28	1.96
dhqMe5Ac37	**17**	5.37	5.65	11.9	6.52/6.41	7.04	6.85	6.88	3.85	2.26	8.17	1.95
dhqMe53′Ac374′	**18**	5.55	5.75	12.1	6.54/6.44	7.40	7.12	7.16	3.86	2.24	3.86/2.26	1.96
dhqMe54′Ac373′	**19**	5.49	5.68	12.1	6.55/6.45	7.28	7.16	7.45	3.85	2.24	2.26/3.85	1.97
dhqMe57Ac33′4′	**20**	5.50	5.57	12.1	6.26/6.22	7.45	7.33	7.51	3.84	3.88	2.28	1.97
cis-dhqMe57Ac33′4′	**20a**	5.82	5.54	2.8	6.28/6.25	7.42	7.26	7.43	3.84	3.89	2.26	1.91
dhqMe73′Ac34′	**21**	5.57	5.96	11.9	6.12/6.13	7.39	7.13	7.16	11.51	3.88	3.86/2.25	1.98
dhqMe74′Ac33′	**22**	5.50	5.87	11.9	6.12/6.13	7.28	7.17	7.44	11.51	3.87	2.25/3.85	1.97
dhqMe57Ac3′4′	**24**	5.11	4.41	11.7	6.25/6.19	7.48	7.31	7.51	3.86	3.87	2.28	4.41
dhqMe5Ac73′4′	**25**	5.22	4.54	12.4	6.54/6.44	7.49	7.31	7.52	3.88	2.26	2.27/2.28	4.54
dhqMe3′Ac374′	**26**	5.66	6.08	12.1	6.37/6.38	7.42	7.13	7.17	11.37	2.30	3.91/2.26	1.98
dhqMe4′Ac373′	**27**	5.61	6.00	12.1	6.36/6.37	7.29	7.18	7.47	11.37	2.29	2.26/3.94	1.99
dhqMe5	**28**	4.85	4.34	11.9	6.16/6.01	7.05	6.84	6.88	3.82	9.85	8.16/8.11	4.26
dhqMe5[b]	**28**	4.82	4.33	11.8	6.11/5.95	6.99	6.81	6.81	3.80			
dhqMe5 (ref 3)		4.86	4.68	12.1	6.14/5.98	6.83	7.03	6.85	3.81			
dhqMe57	**29**	4.89	4.38	11.9	6.21/6.12	7.06	6.85	6.90	3.85	3.86	8.08/8.02	4.26
dhqMe5Ac3	**30**	5.25	5.53	11.8	6.17/6.04	7.01	6.85	6.85	3.80	9.68	8.16	1.94
cis-dhqMe5Ac3	**30a**	5.56	5.48	3.2	6.15/6.10	6.98	6.79	6.83	3.79			1.95

[a] Solvent: acetone-d_6. R = H, Me, or Ac; $J_{2'6'}$ = 1.8−2.1 Hz, $J_{5'6'}$ = 8.0−9.0 Hz, J_{68} = 1.9−2.3 Hz. [b] In acetone-d_6/D_2O (4:1).

Kasai et al.[13] reported H-2 and H-3 chemical shift increments of comparable magnitude for the *trans*- and *cis*-diastereomers of dhq 3-*O*-rhamnoside; the values for the 2*R*,3*R* and 2*S*,3*S* and for the 2*R*,3*S* and 2*S*,3*R* stereoisomers, respectively, differed by considerably smaller amounts (−0.10 to −0.12 ppm for H-2, +0.05 to −0.10 ppm for H-3). The chemical shifts of the A- and B-ring protons of their samples, recorded in acetone-d_6 in the presence of D_2O, are within 0.10 ppm of our values for **1/1a** and **9/9a**, recorded in acetone-d_6 without D_2O. This is also true for the spectral parameters of dhqMe5 (**28**) in the presence versus absence of D_2O (Table 1); that is, the effect of water appears to be insignificant. The 3-OH proton of the *cis*-isomers is additionally deshielded ($\Delta\delta$ 0.4−0.5 ppm) by the anisotropy cone of the carbonyl group, and its vicinal coupling to H-3 is relatively large (6 Hz vs 4 Hz for *trans*); decoupling by

addition of trifluoroacetic acid collapsed the H-3 double doublets to simple doublets. 3′-*O*-Methylation induces a downfield shift of the H-2′ and H-6′ signals ($\Delta\delta$ 0.12−0.15 ppm) but has no effect on H-5′, while 4′-*O*-methylation induces a downfield shift of the H-5′ and H-6′ ($\Delta\delta$ 0.12−0.20 ppm) and a weak upfield shift of the H-2′ signal ($\Delta\delta$ 0.06−0.09). The five aromatic protons are virtually unaffected by the C-ring geometry. We believe that our NMR data, especially the consistency of the chemical shift variations resulting from minor structural changes, prove the structures assigned to the four di-*O*-methyldhqs **10**, **10a**, **11**, and **11a**. Unfortunately, our failure to isolate pure samples precluded confirmation of the absolute stereochemistry (assumed to be 2*R*,3*R* for **10** and **11** and 2*S*,3*R* for **10a** and **11a**) by CD spectroscopy.

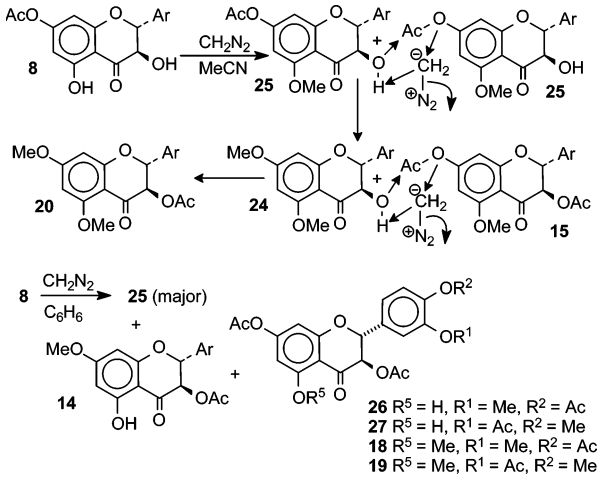
Partially acetylated dihydroflavonols undergo not only *O*-methylation but also ester cleavage (*trans*-esterification) on treatment with CH_2N_2, as previously reported for simple phenol esters.[23,24] Thus dhq 3,5,3′,4′-tetraacetate (**5**) gave not only the expected dhqMe7Ac353′4′ (**13**) but also some dhqMe7Ac33′4′ (**14**), the product of 5-OAc methanolysis (Scheme 1b). Similarly, the 5-*O*-methyl ether **15** formed from the isomeric dhq 3,7,3′,4′-tetraacetate (**6**) was accompanied by five minor byproducts (Scheme 1c) resulting from solvolysis of the phenolic ester functions (7-OAc → 7-OH, **16**, and 3′/4′-OAc → 3′/4′-OH, **17**) and simultaneous or subsequent methylation (3′-OAc → 3′-OMe, **18**, 4′-OAc → 4′-OMe, **19**, and 7-OAc → 7-OMe, **20**).

As expected from these results for the dhq tetraacetates **5** and **6**, dhq 3,3′,4′-triacetate (**7**) reacts with diazomethane in MeOH slowly and exclusively at the 7-OH group; 80% of the educt was recovered after 3 days at 4 °C. Changing the solvent to benzene led to complete reaction and formation of dhqMe7Ac33′4′ (**14**) in 88% yield as well as small amounts of dhqMe73′Ac34′ (**21**), dhqMe74′Ac33′ (**22**), and dhqMe57Ac33′4′ (**20**); that is, a few B-ring acetyl groups were again replaced by methyl groups (Scheme 1d). The presence of some *cis*-dhqMe7Ac33′4′ (**14a**) and *cis*-dhqMe57Ac33′4′ (**20a**) was accounted for by methylation of the (∼15%) *cis*-dhqAc33′4′ (**7a**) formed by epimerization of the *trans*-isomer during its preparation from **6**.[18] We found no evidence for *trans* → *cis* isomerization under the mild experimental conditions used for methylation.

The knowledge gained from the above model reactions, combined with our experience on the deacetylation of dhq acetates,[18] suggested dhq 7,3′,4′-triacetate (**8**) as the most suitable substrate for a short synthesis of dhqMe5. Unexpectedly, its methylation in acetonitrile led to the formation of dhqMe57Ac3′4′ (**24**) and dhqMe57Ac33′4′ (**20**) as major products, together with smaller amounts of dhqMe5Ac373′4′ (**15**) and dhqMe5Ac73′4′ (**25**). This outcome can be rationalized by an intermolecular acetyl migration[25] from the oxygen at C-7 of the initially formed **25** to the more nucleophilic oxygen at C-3, with concomitant methylation at O-7 (Scheme 2). In benzene, acetyl migration was found to be less significant and **25** was formed as the main product, together with five byproducts (**14**, **26**, **27**, **18**, and **19**) resulting from acetyl migration to O-3 from O-7 of **8** and from O-3′ and O-4′ of **8** and **25**, respectively, followed by methylation (Scheme 2). Finally, the aromatic ester functions were cleaved by sodium sulfite in aqueous methanol,[18] providing dhqMe5 (**28**) from dhqMe5Ac73′4′ (**25**), dhqMe57 (**29**) from dhqMe57Ac3′4′ (**24**), and dhqMe5Ac3 (**30**) and its *cis*-diastereomer (**30a**) from dhqMe5Ac373′4′ (**15**) (Scheme 3).

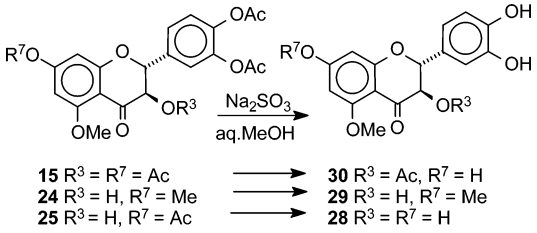
Foo[3] extracted dhqMe5 (**28**) from the heartwood of *Acacia melanoxylon* and characterized it by UV and NMR spec-

Scheme 2. Methylation of Dihydroquercetin 7,3′,4′-triacetate (**8**)[a]



[a] Ar = 3′,4′-$(OAc)_2C_6H_3$.

Scheme 3. Deacetylation of dhqMe5Ac373′4′ (**15**), dhq57Ac3′4′ (**24**), and dhqMe5Ac73′4′ (**25**)



troscopy. Pistelli et al.[4] isolated it from the aerial parts of *Genista corsica* and presented no experimental data to support their structure assignment. The proton NMR spectrum (in acetone-d_6) of our synthetic sample shows low-field aromatic proton signals at δ 7.05 (doublet, *J* 1.9 Hz, H-2′), 6.84 (doublet, *J* 8.1 Hz, H-5′), and 6.88 ppm (double doublet, *J* 1.9 and 8.1 Hz, H-6′), typical of a 3′,4′-dihydroxylated B-ring, the C-ring protons at δ 4.85 (doublet, *J* 11.9 Hz, H-2) and 4.34 ppm (double doublet, *J* 11.9 and 2.6 Hz, H-3), and the OH protons at δ 9.85 (7-OH), 8.16/8.11 (3′,4′-OH), and 4.24−4.34 ppm (doublet, *J* 2.6 Hz, 3-OH); see Table 1, which also shows the data recorded in acetone-d_6/D_2O (4:1) and the previously reported[3] chemical shifts. The hydroxylic protons were cleanly separated by addition of cadmium nitrate.[26] The H-3 signal, which is easily overlooked due to its overlap with 3-OH, experiences an upfield shift relative to dhq (from 4.60 to 4.34 ppm) because the deshielding effect of the 5-OH···O═C< hydrogen bond is nullified on methylation of the 5-OH group. Several additional examples from the NMR table, e.g., **2** versus **3**, **9** versus **29**, **14** versus **20**, **26** versus **18**, and **27** versus **19**, demonstrate the generality of this effect. Cross-peaks in the NOESY spectrum at 8.16/6.84, 8.11/7.05, and 6.16/3.82 ppm verify the chemical shifts assigned to OH-4′/H-5′, OH-3′/H-2′, and H-6/MeO-5, respectively, as well as O-5 as the methylation site (the H-8 signal at 6.01 ppm is not enhanced by saturation of the OMe protons). The ^{13}C signals of C-6 (93.9 ppm) and C-8 (96.3 ppm) were identified by correlation with the proton signals at δ 6.16 and 6.01 ppm, respectively.

In summary, in the presence of strong base Me_2SO_4 converts dhq and its pentaacetate mainly to dhqMe73′4′ and dhqMe573′4′. CH_2N_2 is a more suitable methylating agent for the preparation of mono- and di-*O*-methyldihydroquercetins and their acetates. It permits a limited control of regiochemistry by appropriate choice of educt and

solvent. The 7-OH is most and the 5-OH least reactive, while 3-OH is usually inert. In methanol the H-bonded 5-OH is also inert, permitting exclusive 7-*O*-methylation when 3′,4′-OH are protected. 5-*O*-Methylation succeeds in aprotic solvents (acetonitrile or benzene) and is followed by *trans*-esterification if an electron-rich oxygen, such as 3-OH, is available to accept the migrating acetyl group. DhqMe5 has been synthesized from dhq by taking advantage of these reactivity differences.

Experimental Section

General Experimental Procedures. Dihydroquercetin (**1**, dhq) was extracted from Douglas fir bark.[22,27] The partial dhq acetates dhqAc353′4′ (**5**), dhqAc373′4′ (**6**), dhqAc33′4′ (**7**), dhqAc73′4′ (**8**), and dhqAc3 (**4**) were prepared by acetylation of dhq as described previously.[18] Dimethyl sulfate (Aldrich) was purified by extraction with ice water and 0.5 M $NaHCO_3$ and dried over anhydrous Na_2SO_4. Melting points (uncorrected) were recorded on a Fisher-Johns melting point apparatus, 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). Evaporations were carried out on a water bath (70−80 °C) 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 eluant, unless specified otherwise. Elemental analyses were run on a Carlo-Erba elemental analyzer model 1106.

Methylation of dhq (1/1a) with CH_2N_2. An ethereal solution of diazomethane generated from 105 mg of *N*-nitrosomethylurea (1.02 mmol) and 0.5 mL of 40% KOH[28] was added to a solution of an equimolar mixture (65 mg, 0.21 mmol) of *trans*- and *cis*-dhq (**1** and **1a**)[22] in 3 mL of Et_2O and 1 mL of benzene. The yellow reaction mixture was stored at 4 °C for 9 days. Solvent evaporation and air-drying at 80 °C left a yellow solid residue (70 mg), which was found by PTLC and ^{1}H NMR analysis of each fraction to contain dhq (**1/1a**, R_f 0.22, *trans*/*cis* 2:3), dhqMe7[29] (**9/9a**, R_f 0.34, *trans*/*cis* 5:3), dhqMe73′ (**10/10a**, R_f 0.52, *trans*/*cis* 2:1), dhqMe74′ (**11/11a**, R_f 0.52, *trans*/*cis* 2:1), and dhqMe73′4′ (**2/2a**, R_f 0.65, *trans*/*cis* 4:1) in the approximate molar ratio 2:10:3:3:1 (overall yield 78%). ^{1}H NMR, Table 1; ^{13}C NMR (Me_2CO-d_6), *cis*-dhqMe7 (**9a**): δ 196.7 (C-4), 169.0/165.3 (C-5/7), 163.8 (C-8a), 146.1/145.6 (C-3′/4′), 128.5 (C-1′), 120.1 (C-6′), 102.2 (C-4a), 95.5/94.5 (C-6/8), 82.4 (C-2), and 72.7 (C-3). When a solution of 32.2 mg of pure *trans*-dhq (**1**, 0.106 mmol) and 15.3 mg of $NaBO_2 \cdot 4H_2O$ (0.111 mmol) in 2 mL of MeOH was treated with ethereal CH_2N_2 (3 days at 4 °C) by the same procedure, diluted with water, neutralized with 0.1 mL of 1 M HCl, extracted with EtOAc, and dried ($MgSO_4$), the evaporation residue (26.6 mg, 82%) was found to contain only *trans*-dhqMe7 (**9**) and dhq (ratio 1:7).

Padmatin 3-Acetate (12, dhqMe7Ac3). Application of the methylation procedure described above (CH_2N_2 from 1.03 mmol of *N*-nitrosomethylurea) to a solution of 57 mg of dhqAc3 (**4**, 0.165 mmol) in 1.5 mL of MeOH (27 h at 4 °C) gave 17 mg of **12** (30% yield). ^{1}H NMR in $CDCl_3$ was consistent with literature;[20a] most of the educt was recovered.

7-*O*-Methyldihydroquercetin Tetraacetate (13, dhqMe7Ac353′4′).[12,20] Methylation of a solution of 40 mg (0.085 mmol) of dhqAc353′4′(**5**)[18] in 3 mL of benzene/MeOH (1:1) with excess ethereal CH_2N_2 (3 days at 4 °C) gave 22.6 mg of crude product (55% yield), which was separated by PTLC. Three bands were eluted: R_f 0.68 (10.7 mg) dhqMe7Ac353′4′ (**13**), ^{1}H NMR (Me_2CO-d_6), Table 1; R_f 0.49 (2.4 mg) unreacted starting material (**5**); R_f 0.74 (2.3 mg) dhqMe7Ac33′4′ (**14**, ^{1}H NMR, Table 1).

5-*O*-Methyldihydroquercetin Tetraacetate (15, dhqMe5Ac373′4′). Methylation of a solution of 196 mg (0.415 mmol) of dhqAc373′4′ (**6**)[18] in 4 mL of benzene with excess ethereal CH_2N_2 (4 days at 4 °C) gave 184 mg of crude product (91% yield), which was purified by PTLC (blue fluorescent band at R_f 0.57). **15:** ^{1}H NMR, see Table 1; NOESY correlations: H-6 ↔ MeO-5/AcO-7, H-8 ↔ H-2/AcO-7, H-3 ↔ H-2′/AcO-3, H-2′ ↔ H-2/AcO-3/AcO-3′, and H-5′ ↔ H-6′/AcO-4′; ^{13}C NMR (Me_2CO-d_6) δ (ppm) 184.7 (C-4), 169.4 (3-OAc), 168.8 (7-OAc), 168.6 (3′,4′-OAc), 163.6 (C-7), 162.8 (C-8a), 158.2 (C-5), 144.0/143.3 (C-3′,4′), 135.6 (C-1′), 126.3 (C-6′), 124.6/123.9 (C-2′/5′), 108.3 (C-4a), 104.0 (C-6), 100.5 (C-8), 80.6 (C-2), 74.5 (C-3), 56.7 (OMe), 21.0 (7-OAc), 20.5 (3′,4′-OAc), and 20.3 (3-OAc). The following minor byproducts were isolated from three weak PTLC bands and identified by ^{1}H NMR (Table 1): 5-*O*-methylquercetin tetraacetate[25ab] (R_f 0.30) ^{1}H NMR (Me_2CO-d_6): δ 7.89 (dd, *J* 2.1/8.6 Hz, H-6′), 7.85 (d, H-2′), 7.46 (d, H-5′), 7.07 (d, *J* 2.0 Hz, H-8), 6.82 (d, H-6), 3.92 (s, OMe), 2.31, 2.28−2.26 (12H, OAc); dhqMe7Ac33′4′ (**14**), dhqMe5Ac33′4′ (**16**), dhqMe5Ac37 (**17**) (R_f 0.36); dhqMe57Ac33′4′ (**20**), dhqMe53′Ac374′ (**18**), and dhqMe54′Ac373′ (**19**) (R_f 0.61).

7-*O*-Methyldihydroquercetin 3,3′,4′-Triacetate (14, dhqMe7Ac33′4′). DhqAc33′4′, prepared by refluxing a methanolic solution of dhqAc373′4′ (**6**) for 47 h, followed by PTLC (R_f 0.55) of the crude product,[18] was found (by ^{1}H NMR analysis) to be a mixture of the *trans*-(**7**) and the *cis*-isomer (**7a**), molar ratio 6:1. A solution of 40 mg (0.093 mmol) of this mixture in 2 mL of MeOH was methylated with excess ethereal CH_2N_2 (3 days at 4 °C). The crude product (30.4 mg) consisting of 20% **14** and 80% **7/7a** was then dissolved in 1 mL of benzene and 1 mL of ether and methylated again (3 days at 4 °C). The yellow solid (27.6 mg) left behind after solvent evaporation contained no more unreacted starting material. It was recrystallized from MeOH to give dhqMe7Ac33′4′ (**14**), white needles, mp 180−1 °C. Anal. Calcd: C 59.70, H 4.71 (calcd for $C_{22}H_{20}O_{10}$: C 59.46, H 4.54). Three fractions were separated by PTLC of the concentrated mother liquor: R_f 0.84 (1.9 mg), equimolar mixture of dhqMe73′Ac34′ (**21**) and dhqMe74′Ac33′ (**22**); R_f 0.77 (11.7 mg), **14** and 10% *cis*-dhqMe7Ac33′4′ (**14a**); R_f 0.51 (2.0 mg, blue fluorescent), dhqMe57Ac33′4′ (**20**), and 5% *cis*-dhqMe57Ac33′4′ (**20a**); ^{1}H NMR, see Table 1.

Methylation of Dihydroquercetin 7,3′,4′-Triacetate (8, dhqAc73′4′). A solution of 45.9 mg (0.107 mmol) of dhqAc73′4′-(**8**)[18] in 2 mL of MeCN was methylated with excess ethereal CH_2N_2 (4 days at 4°C). The two major bands obtained by PTLC of the crude product (46.2 mg) were collected and analyzed by ^{1}H NMR: R_f 0.49 (10.2 mg) dhqMe57Ac33′4′ (**20**)/dhqMe5Ac733′4′ (**15**) (molar ratio 4:1) and R_f 0.36 (9.4 mg) dhqMe57Ac3′4′ (**24**)/dhqMe5Ac73′4′ (**25**) (7:1); ^{1}H NMR, see Table 1. Methylation of 90.0 mg (0.209 mmol) of dhqAc73′4′-(**8**) in 8 mL of benzene (1 h/4 °C) gave 99.0 mg of a 3:1 mixture of unreacted educt and dhqMe5Ac73′4′ (**25**), which was methylated again with excess ethereal CH_2N_2 (14 h/4 °C). PTLC (elution with toluene/EtOAc, 7:3) of the crude product (86.8 mg) afforded 26.0 mg of **25** (R_f 0.18); ^{13}C NMR (Me_2CO-d_6) δ (ppm) 191.2 (C-4), 168.9 (7-OAc), 168.6 (3′,4′-OAc), 164.0 (C-7), 162.5 (C-8a), 158.3 (C-5), 143.7/143.3 (C-3′/4′), 136.8 (C-1′), 126.8 (C-6′), 124.2/123.7 (C-2′/5′), 107.6 (C-4a), 104.0 (C-6), 100.1 (C-8), 83.1 (C-2), 73.8 (C-3), 56.7 (OMe), 21.0 (7-OAc), and 20.5 (3′,4′-OAc). The following minor byproducts were detected in the ^{1}H NMR spectra of the more mobile PTLC fractions: dhqMe3′Ac374′ (**26**) and dhqMe4′Ac373′ (**27**) at R_f 0.63, dhqMe7Ac33′4′ (**14**) at R_f 0.57, dhqMe53′Ac374′ (**18**) and dhqMe54′Ac373′ (**19**) at R_f 0.35.

5-*O*-Methyldihydroquercetin 3-Acetate (30, dhqMe5Ac3). A solution of 41.3 mg (0.328 mmol) of sodium sulfite in 2 mL of water was added dropwise to a stirred solution of 51.0 mg (0.105 mmol) of dhqMe5Ac373′4′ (**15**) in 2 mL of methanol. The clear yellow reaction mixture was stirred for 22 h at 24 °C. DhqMe5Ac3 (**30**) crystallized (21.7 mg of white solid after filtration, washing, and vacuum-drying over P_2O_5) on evaporation of the MeOH, mp 214−6 °C (from acetone). Anal. Calcd: C 60.23, H 4.54 (calcd for $C_{18}H_{16}O_8$, C 60.00, H 4.48). ^{13}C NMR (Me_2CO-d_6): δ (ppm) 184.3 (C-4), 169.5 (OAc), 165.8 (C-5), 164.9/163.8 (C-7/8a), 146.8/145.9 (C-3′,4′), 128.9 (C-1′), 120.5 (C-6′), 115.9/115.3 (C-2′/5′), 96.6 (C-8), 94.4 (C-6), 81.5 (C-2), 74.5 (C-3), 56.1 (OMe), and 20.4 (OAc). The chemical shifts of H-6 (6.17 ppm) and H-8 (6.04 ppm) were distinguished by NOESY (H-6/OMe cross-peak at δ 6.17/3.80),

those of C-6 (94.4) and C-8 (96.6) by C-H COSY. An additional fraction of crude product (10.3 mg), obtained by two consecutive extractions (before and after acidification with HCl) of the aqueous filtrate with EtOAc, drying ($MgSO_4$), and solvent evaporation, was found to contain **30**, *cis*-dhqMe5Ac3 (**30a**), and dhqMe5 (**28**) in the approximate molar ratio 6:4:1 (combined yield of 72% **30** and 10% **30a**); ^{1}H NMR, Table 1.

5-*O*-Methyldihydroquercetin (28, dhqMe5) and 5,7-Di-*O*-methyldihydroquercetin (29, dhqMe57). A solution of 39 mg (0.31 mmol) of sodium sulfite in 2.5 mL of water was added dropwise to a stirred solution of 25.7 mg (0.058 mmol) of dhqMe5Ac73′4′ (**25**) in 2.5 mL of methanol. The clear yellow reaction mixture was stirred for 16 h at 23 °C, then acidified with 0.3 mL of 1 M HCl. Methanol evaporation, extraction with EtOAc, drying ($MgSO_4$), and solvent evaporation gave 11.3 mg of dhqMe5 (**28**), colorless crystals (61% yield), mp 244−5° (from acetone/water) (lit.[3] 251−3 °C). Anal. Calcd: C 60.54, H 4.57 (calcd for $C_{16}H_{14}O_7$, C 60.38, H 4.43). ^{13}C NMR (Me_2CO-d_6/D_2O): δ (ppm) 191.5 (C-4), 166.3/165.1 (C-5/7), 163.3 (C-8a), 146.2/145.4 (C-3′/4′), 129.3 (C-1′), 120.4 (C-6′), 115.7/115.6 (C-2′/5′), 102.7 (C-4a), 96.3 (C-8), 93.9 (C-6), 83.6 (C-2), 73.3 (C-3), and 56.1 (OMe). Deacetylation of a 7:1 mixture of dhqMe57Ac3′4′ (**24**) and dhqMe5Ac73′4′ (**25**) (9.4 mg, R_f 0.36) (see above: methylation of dhqAc73′4′ in MeCN) by the same procedure gave **28** as minor product and dhqMe57 (**29**); ^{1}H NMR, see Table 1. On addition of trifluoroacetic acid, the δ 4.26 doublet (J 2.6 Hz, 3-OH) shifted off-range and the δ 4.38 double doublet (J 11.9 and 2.6 Hz, H-3) collapsed to a doublet.

Acknowledgment. We thank the Dean of Science for financial support and Ms. Tracey for recording the NMR spectra.

References and Notes

(1) In the abbreviated names, such as dhqMe7Ac3 for 7-*O*-methyldihydroquercetin 3-acetate (**12**), the numbers conform to standard flavonoid nomenclature (see Scheme 1, structure of dhq) and designate the positions of the oxygen atoms of dihydroquercetin (dhq), to which the methyl (Me) and acetyl (Ac) substituents are attached.
(2) (a) Bohm, B. A. In *The Flavonoids*; Harborne, J. B., Mabry, T. J., Mabry, H., Eds.; Chapman and Hall: London, 1975; Chapter 11, pp 591−592. (b) Bohm, B. A. In *The Flavonoids: Advances in Research since 1980*; Harborne, J. B., Ed.; Chapman and Hall: London, 1988; Chapter 9, pp 378 and 383. (c) Bohm, B. A. In *The Flavonoids: Advances in Research since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994; Chapter 9, p 424.
(3) Foo, L. Y. *Phytochemistry* **1987**, *26*, 813−817.
(4) Pistelli, L.; Giachi, I. *J. Nat. Prod.* **2000**, *63*, 504−506.
(5) Pavanasasivam, G.; Sultanbawa, M. U. S. *J. Chem. Soc., Perkin Trans. 1* **1975**, 612−613.
(6) Marco, J. A.; Sanz-Cervera, J. F.; Yunte, A.; Oriola, M. C. *Phytochemistry* **1994**, *36*, 725−729.
(7) Ruangrungsi, N.; Tappayuthpijarn, P.; Tantivatana, P.; Borris, R. P.; Cordell, G. A. *J. Nat. Prod.* **1981**, *44*, 541−545.
(8) Ferreira, V. F.; Parente, J. P.; Pinto, M. C. F. R.; Pinto, A. V.; Porter, B.; Silva, M. M.; Moutinho, J. L. *Biochem. Syst. Ecol.* **1986**, *14*, 199−202.
(9) Islam, M. T.; Tahara, S. *Phytochemistry* **2000**, *54*, 901−907.
(10) Zdero, C.; Bohlmann, F.; King, R. M.; Robinson, H. *Phytochemistry* **1986**, *25*, 2841−2855.
(11) Bohlmann, F.; Czerson, H.; Schöneweiss, S. *Chem. Ber.* **1977**, *110*, 1330−1334.
(12) Marambio, O.; Silva, M. *Bol. Soc. Chil. Quim.* **1989**, *34*, 105−113.
(13) Kasai, R.; Hirono, S.; Chou, W.-H.; Tanaka, O.; Chen, F.-H. *Chem. Pharm. Bull.* **1988**, *36*, 4167−4170.
(14) Nanayakkara, N. P. D.; Hussain, R. A.; Pezzuto, J. M.; Soejarto, D. D.; Kinghorn, A. D. *J. Med. Chem.* **1988**, *31*, 1250−1253.
(15) Tückmantel, W.; Kozikowski, A. P.; Romanczyk, L. J., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 12073−12081.
(16) Hergert, H. L.; Coad, P.; Logan, A. V. *J. Org. Chem.* **1956**, *21*, 304−310.
(17) Porter, L. J.; Foo, L. Y. *Phytochemistry* **1982**, *21*, 2947−2952.
(18) Kiehlmann, E. *Org. Prep. Proceed. Int.* **1999**, *31*, 87−97.
(19) Slabbert, N. P. *Tetrahedron* **1977**, *33*, 821−824.
(20) (a) Grande, M.; Piera, F.; Cuenca, A.; Torres, P.; Bellido, I. S. *Planta Med.* **1985**, 414−419. (b) Wollenweber, E.; Mayer, K.; Roitman, J. N. *Phytochemistry* **1991**, *30*, 2445−2446.
(21) (a) Timmermann, B. N.; Hoffmann, J. J.; Shivanand, D. J.; Bates, R. B.; Siahaan, T. J. *Phytochemistry* **1986**, *25*, 723−727. (b) Rauter, A. P.; Branco, I.; Tostao, Z.; Pais, M. S.; Gonzalez, A. G.; Bermejo, J. B. *Phytochemistry* **1989**, *28*, 2173−2175.
(22) Kiehlmann, E.; Li, E. P. M. *J. Nat. Prod.* **1995**, *58*, 450−455.
(23) Nierenstein, M. *J. Am. Chem. Soc.* **1930**, *52*, 4012−4013.
(24) Bredereck, H.; Sieber, R.; Kamphenkel, L. *Chem. Ber.* **1956**, *89*, 1169−1176.
(25) (a) Kubota, O.; Perkin, A. G. *J. Chem. Soc.* **1925**, *127*, 1889−1896. (b) Jurd, L.; Rolle, L. A. *J. Am. Chem. Soc.* **1958**, *80*, 5527−5531. (c) Simpson, T. H.; Wright, R. S. *J. Org. Chem.* **1961**, *26*, 4686−4689.
(26) Suzuki, Y.; Anazawa, I. *Bunseki Kagaku* **1982**, *31*, 498−503.
(27) Hergert, H. L. In *The Chemistry of Flavonoid Compounds*; Geissman, T. A., Ed.; MacMillan: New York, 1962; Chapter 17, pp 575−579.
(28) (a) Arndt, F. *Organic Syntheses*; Wiley: New York, 1943; Collect. Vol. 2, pp 165−167. (b) Arndt, F.; Amstutz, E. D.; Myers, R. R. *Organic Syntheses*; Wiley: New York, 1943; Collect. Vol. 2, pp 461−464.
(29) Öksüz, S.; Topçu, G. *Phytochemistry* **1992**, *31*, 195−197.

NP034005W