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Epimerization, transacylation and bromination of dihydroquercetin acetates; synthesis of 8-bromodihydroquercetin

Research Article

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Abstract: Dihydroquercetin (*dhq*) and its 3-acetate react with acetic anhydride in the absence of a base catalyst to yield mixtures of partially acetylated products. Three new esters were characterized by NMR spectroscopy as *dhq* 3,7,3'-triacetate, 3,7,4'-triacetate and 5,7,3',4'-tetraacetate. At its melting point neat *dhq* 3,7,3',4'-tetraacetate is partially converted to *dhq* 3,3',4'-triacetate and *dhq* pentaacetate by intermolecular acetyl transfer. *Dhq* 7,3',4'-triacetate yields exclusively *dhq* 3',4'-di- and 3,7,3',4'-tetraacetate under these conditions. The acetylation/deacetylation reactions are accompanied by partial epimerization: 3 new acetates with 2,3-*cis* stereochemistry (*dhq* 3-, 3,7,3',4'-tetra- and penta-) were identified. *Dhq* and its 3,7,3',4'-tetraacetate undergo regiospecific dibromination at C-6 and C-8 with excess *N*-bromosuccinimide in polar solvents, and 6,8-dibromo-*dhq* can be regioselectively debrominated to 8-bromo-*dhq* with sodium sulfite.

Keywords: *Dihydroflavonol acetylation • Cis-2,3-dihydroflavonols • 6,8-dibromoflavanonols • Taxifolin • Intermolecular acetyl transfer*

1. Introduction

Flavonoids are ubiquitous in the plant kingdom. They occur primarily as free phenols, glycosides, methyl ethers or acetates and fulfill essential physiological functions (see Abbreviations) [1]. One of the most abundant dihydroflavonols, dihydroquercetin (*dhq*, **1,** also called *taxifolin*), has attracted wide-spread interest as food additive and in cosmetics because of its radical-scavenging (antioxidant) properties. It can be extracted from Douglas fir bark, a major waste product of the forest industry in the Pacific Northwest [2], and is extracted on a commercial scale [3] from Mongolian larch wood. *Dhq* 3-acetate (*dhqAc3*, **2**), a constituent of young *Tessaria dodoneifolia* shoots, is eighty times sweeter than sucrose [4,5]. It can be prepared by exhaustive *dhq* acetylation and subsequent partial deacetylation of the pentaacetate [6]. Expecting that other *dhq* acetates might also exhibit unusual bioactivity, we identified 22 partially acetylated intermediates (of 30 possible structures) formed in the reaction of *dhq* with acetic anhydride, as reported previously [6]. Preparation of the eight elusive acetates by further variation of the acetylation conditions and their characterization by ^{1}H NMR was the main objective of the research described herein. Concurrently we decided to search for routes to monobrominated dihydroflavonols which are desirable starting materials for the synthesis of procyanidins and condensed tannins.

2. Experimental Procedure

Aceticanhydride(Ac_2O),pyridineand*N*-bromosuccinimide (NBS) were purchased from Sigma-Aldrich and used without further purification. Dihydroquercetin (*dhq*) was extracted from Douglas fir bark [2] and converted to *dhqAc*$_5$ (**7**), *dhqAc373'4'* (**5**), *dhqAc73'4'* (**11**) and *dhqAc3* (**2**) as described previously [6]. NMR spectra were recorded 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), or to the chloroform resonances at 7.26 (^{1}H NMR) and 77.0 ppm (^{13}C NMR). Solvent evaporations were carried out on a water bath (70-80°) or at reduced pressure on a rotary evaporator. Preparative thin layer chromatography (PTLC) was performed on Merck Kieselgel 60HF-254/366 (0.75 mm) plates with benzene/acetone (4:1 v/v) as eluant. Low-resolution mass spectra were run on an HP-5985 quadrupole instrument.

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2.1. Acetylation of dihydroquercetin (1)

A solution of 4.4 mg *dhq* (14.5 µmol) in 0.50 mL Ac_2O (6.0 mmol) was heated to 80° for one hour. Evaporation to dryness (80°) left 7.7 mg of a beige solid containing *dhqAc*$_5$ (**7** and **7c**) and the four tetraacetates *dhqAc373'4'* (**5**), *dhqAc573'4'* (**6**), quercetin 3,7,3',4'-tetraacetate and *cis-dhqAc373'4'* (**5c**) in the approximate molar ratio 12:12:4:3:1.5 (analysis by ^{1}H NMR, see Table 1). The products were separated by PTLC.

2.2. DhqAc3' (3) and dhqAc4' (4)

Acetic anhydride (5.0 mL, 53 mmol) was added in two portions to 76.1 mg *dhq* (0.25 mmol) in a porcelain dish and allowed to evaporate in the fumehood (25°/7 h). PTLC of the light brown solid residue (102 mg) afforded 31 mg of a 1:1 mixture of *dhqAc3'* (**3**) and *dhqAc4'* (**4**) (R_f 0.36), smaller amounts of *dhqAc3'4'* (**12**), *dhqAc73'* and *dhqAc74'* (R_f 0.42) and unreacted starting material (R_f 0.25). Compounds **3** and **4**: ^{1}H NMR [6], NOESY cross peaks at 8.69/7.00 and 8.62/7.19 ppm, ^{13}C NMR (Table 2).

2.3. DhqAc373' (9), dhqAc374' (8) and cis-dhqAc3 (2c).

As described previously [6], *dhqAc*$_5$ (**7**) was partially deacetylated with sodium sulfite in 50% aqueous MeOH. Gel permeation chromatography (Sephadex LH-20, elution with 95% EtOH) of the crude product removed the minor byproducts but did not separate the two diastereomers of *dhqAc3* (**2** and **2c**); neither did PTLC or flash chromatography on silica followed by elution with benzene/Me_2CO (4:1). On reaction with 1.0 mL Ac_2O (25°/12 h) and solvent evaporation, the fraction (4.1 mg) with the highest concentration of 2,3-*cis* isomer (*trans/cis* ratio 5:1) gave 5.3 mg of a mixture of *dhqAc373'4'* (**5**), *dhqAc373'* (**9**), *dhqAc374'* (**8**), *cis-dhqAc373'4'* (**5c**), *dhqAc33'4'* (**10**) (molar ratio 10:2:2:1:1, analysis by ^{1}H NMR) as well as traces of *dhqAc33'* and *dhqAc34'*.

2.4. Transacylation of dhqAc73'4' (11) and dhqAc373'4' (5).

A 4-mg sample of pure *dhqAc73'4'* (**11**) was heated to 142° for four hours in a capillary melting point tube. Subsequent ^{1}H NMR analysis revealed the presence of *dhqAc373'4'* (**5**), *dhqAc3'4'* (**12**) (1:1 molar ratio), *dhqAc33'4'* (**10**) and unreacted starting material. No *dhqAc573'4'* (**6**) was detected. A similar experiment with *dhqAc373'4'* (**5**) (149°/4 h) resulted in its partial conversion to *dhqAc*$_5$ (**7**) and *dhqAc33'4'* (**10**) (1:1) and the formation of some *cis-dhqAc373'4'* (**5c**).

2.5. Bromination of dihydroquercetin

N-Bromosuccinimide (6.4 mg, 0.036 mmol) in 0.5 mL acetone was added dropwise to 5.3 mg of *dhq* (0.018 mmol) in 0.5 mL acetone. Evaporation of the solvent (80°) from the orange solution after 43 min stirring at 25° and succinimide removal by EtOAc/H_2O extraction afforded 8.3 mg of yellow, slightly resinous solid 6,8-dibromodihydroquercetin (**15**) (94% yield) and traces of monobrominated *dhq*. ^{1}H and ^{13}C NMR (Me_2CO-d_6): Tables 1 and 2. With 1.1 equivalents NBS under the same conditions 8-bromodihydroquercetin (**13**), 6-bromodihydroquercetin, **15** and *dhq* were formed in the approximate molar ratio 3:3:2:2 (analysis by ^{1}H NMR, see Table 1).

2.6. 6,8-Dibromodihydroquercetin 3,7,3',4'-tetraacetate (16).

A solution of 39 mg NBS (0.22 mmol) in 0.75 mL dimethylformamide (DMF) was dropped slowly into a solution of 47.1 mg *dhqAc373'4'* (**5**) (0.10 mmol) in 0.5 mL DMF. Stirring for 24 hours, quenching in ice water, filtration and drying (90°) afforded 6,8-dibromo*dhqAc373'4'* (**16**) (beige solid) in 88% crude yield, 53% after two recrystallisations from 95% EtOH; mp 183-8° (lit. 203-4°) [7] depressed by traces of the 2,3-*cis* stereoisomer. EIMS (70 eV): triplets characteristic of the molecular ion at m/z 630 (M^+) and the dibromo fragments 588 (M^+-ketene), 546 (M^+-2·ketene), 528 (M^+-ketene-HOAc), 504 (M^+-3·ketene), 486 (M^+-2·ketene-HOAc), 462 (M^+-4·ketene) and 444 (M^+-3·ketene-HOAc). ^{1}H and ^{13}C NMR (Me_2CO-d_6): Tables 1 and 2. The same compound was obtained in 61% yield by acetylation of 6,8-dibromodihydroquercetin (**15**) (see above) with Ac_2O/pyridine followed by standard workup [6]. Adding only one equivalent of NBS to *dhqAc373'4'*(**5**) under otherwise identical conditions afforded unreacted substrate, 6,8-dibromo*dhqAc373'4'* (**16**), 8-bromo*dhqAc373'4'* (**14**) and the 6-bromo isomer in the molar ratio 6:6:5:1, while in acetone as solvent half of the starting material was dibrominated, only 10% was monobrominated and the rest remained unreacted after one hour reaction time. Dihydroquercetin pentaacetate (**7**) did not react at all. Magnetic stirring of a solution of 6,8-dibromo*dhqAc373'4'* (**16**) in wet DMF for three months led to partial ester hydrolysis forming 6,8-dibromo*dhqAc353'4'* (R_f 0.73) and 6,8-dibromo*dhqAc33'4'* (R_f 0.43) which were separated from **16** (R_f 0.88) by PTLC. The ^{1}H and ^{13}C NMR data of the bromination products are listed in Tables 1 and 2.

Table 1. Proton Chemical Shifts (ppm) of Dihydroquercetin Derivatives

Compound	H-2	H-3	J_{23} [a]	H-6/8	H-2'	H-5'	H-6'	5-OR[b]	3-OR[b]
dhq (1)	5.01	4.60	11.4	5.98/5.94	7.06	6.85	6.90	11.71	4.69
dhqAc374' (8)	5.60	5.99	12.1	6.37/6.38	7.21	7.12	7.09	11.69	2.00
dhqAc373' (9)	5.57	5.98	12.1	6.36/6.37	7.27	7.03	7.35	11.67	1.98
dhqAc573'4' (6)	5.33	4.66	12.1	6.81/6.66	7.50	7.32	7.54	2.30	4.72
cis-dhq [2]	5.41	4.26	2.7	5.99/5.95	7.09	6.80	6.89	11.85	5.14
cis-dhqAc3 (2c)	5.65	5.63	2.9	6.05/6.00	7.00	6.83	6.85	11.7	1.96
cis-dhqAc373'4' (5c)	6.00	5.79	2.6	6.48/6.40	7.46	7.29	7.45	11.47	1.95
cis-dhqAc$_5$ (7c)	5.99	5.63	2.6	6.94/6.70	7.46	7.29	7.45	2.26	1.93
6,8-diBr-dhq (15)	5.25	4.76	11.4	-	7.10	6.87	6.94	12.54	5.03
6,8-diBr-dhqAc373'4' (16)	5.90	6.17	12.3	-	7.50	7.39	7.62	12.09	2.03
6,8-diBr-dhqAc33'4'	5.76	5.98	12.0	-	7.48	7.37	7.59	12.28	2.02
6,8-diBr-dhqAc3573'4'	5.76	5.90	11.8	-	7.50	7.37	7.62	2.38	1.99
8-Br-dhq (13)	5.17	4.67	11.4	6.22	7.10	6.87	6.94	11.76	4.87
6-Br-dhq	5.07	4.66	11.6	6.18	7.10	6.87	6.94	12.47	5.30
8-Br-dhqAc373'4' (14)	5.85	6.10	12.2	6.59	7.50	7.38	7.62	11.40	2.02
6-Br-dhqAc373'4'	5.77	6.08	12.3	6.61	7.50	7.38	7.62	11.40	2.01
8-Br-dhqAc$_5$	5.84	5.94	12.4	6.87	7.53	7.38	7.66	2.29	1.99

Solvent: acetone-d_6; a) Hz; b) R = H or Ac; J_{68} = 2.0-2.2 Hz; $J_{2'6'}$ = 1.9-2.2 Hz; $J_{5'6'}$ = 8.0-8.5 Hz

Table 2. ^{13}C NMR Chemical Shifts (ppm) of Dihydroquercetin Derivatives

Compound	C-2	C-3	C-4	C-4a	C-6/8	C-5/7	C-8a	C-1'	C-2'/5'	C-3'/4'	C-6'
dhq (1)	84.3	73.0	198.0	101.4	97.0/96.0	164.8/167.8	164.0	129.6	115.7	145.6/146.4	120.8
cis-dhq (1c)[a]	82.2	72.5	196.1	101.4	96.7/95.7	165.4/167.4	163.8	128.4	115.4/115.6	145.4/145.8	119.9
dhqAc33'4' (10)	80.8	73.0	192.4	101.9	97.6/96.4	165.1/168.2	163.3	135.3	123.9/124.6	143.3/144.0	126.3
cis-dhqAc33'4' (10c)[b]	80.2	71.0	190.5	102.3	97.6/96.3	165.8/168.2	163.4	134.5	123.2/124.3	143.4/144.0	125.6
dhqAc3 (2)[c]	81.9	73.0	193.0	102.0	97.4/96.3	165.1/168.0	163.8	128.3	115.9/115.4	147.0/145.9	120.6
dhqAc3'(3)[c]	83.7	73.0	197.9	101.4	97.2/96.1	164.9/167.8	163.8	129.7	123.8/117.4	139.3/149.5	127.3
dhqAc4'(4)[c]	83.8	73.1	197.7	101.4	97.1/96.0	164.9/167.8	163.9	136.7	117.3/123.6	150.2/139.9	120.1
dhqAc7[c]	84.6	73.4	200.0	105.4	103.7/102.5	163.2/159.7	163.6	129.3	115.9/115.8	145.7/146.7	120.9
dhqAc35[c]	81.8	73.9	185.6	106.8	106.5/101.9	153.4/165.4	164.5	128.3	115.9/115.4	145.8/146.9	120.5
dhqAc73'[c]	83.9	73.3	199.7	105.4	103.8/102.6	163.0/159.7	163.6	129.4	123.7/117.4	139.4/149.6	127.4
dhqAc74'[c]	84.0	73.4	199.9	105.4	103.9/102.6	163.1/159.7	163.6	136.4	117.4/123.8	150.4/140.0	120.1
dhqAc73'4'[c] (11)[c]	83.4	73.3	199.5	105.3	104.1/102.6	162.8/159.7	163.6	136.5	124.3/123.8	143.8/143.3	126.8
dhqAc353'4'[c]	80.8	73.8	185.0	106.8	106.8/102.0	153.5/165.5	164.2	135.4	123.9/124.6	143.3/144.0	126.4
dhqAc373'4' (5)[d]	81.1	73.4	194.2	105.8	104.5/102.9	162.7/160.2	163.9	134.9	124.0/124.7	143.5/144.3	126.4
dhqAc$_5$ (7)[c]	81.2	74.1	186.0	111.7	112.4/109.8	152.5/157.7	163.4	135.1	124.1/124.7	143.5/144.3	126.4
8-Br-dhq (13)	84.8	72.9	198.2	102.2	97.3/89.2	163.4/164.0	160.1	129.2	115.8	145.7/146.6	120.8
6,8-diBr-dhq (15)	84.9	72.7	198.4	102.5	91.0/89.7	159.6/159.7	158.8	128.9	115.8/115.9	145.7/146.6	120.9
6,8-diBr-dhqAc373'4'(16)[e]	80.8	72.1	192.5	105.8	99.5/97.0	156.8/158.6	154.5	132.6	122.7/124.0	142.2/143.1	125.0

Solvent acetone-d_6; a) [2]; b) [17]; c) see [6] for ^{1}H NMR; d) [30]; e) in $CDCl_3$; δ(MeCOO-) 20.2-20.7/169.3-169.5 ppm

2.7. Partial debromination of 6,8-dibromodihydroquercetin (15).

Dihydroquercetin (520 mg, 1.71 mmol) was brominated with 665 mg NBS (3.74 mmol) in 25 mL acetone (25°/50 min) as described above. The dark yellow, crude **15** (1.174 g, including succinimide and excess NBS) was dissolved in 20 mL MeOH, and a solution of 144 mg $NaHCO_3$ (1.71 mmol) in 10 mL water was added slowly (5 min), followed by dropwise addition (15 min) of a solution of 215 mg (1.71 mmol) Na_2SO_3 in 10 mL water. After stirring the clear orange solution for 30 min, the methanol was evaporated at 35°. Extraction of the aqueous residue with EtOAc (3×20 mL), drying of the organic layer ($MgSO_4$), filtration and solvent evaporation (rotavac) afforded 479 mg of a dark yellow crystalline solid consisting of 8-bromodihydroquercetin (**13**) (no 6-bromo isomer), a trace of its 2,3-*cis* epimer and succinimide. The latter was washed out with water (3×2 mL). A second crop of 181 mg, contaminated with unreacted **15**, was obtained by extraction of the acidified aqueous layer with EtOAc. The 3,7,3',4'-tetraacetate (**14**) of 8-Br*dhq* (**13**) cannot be prepared from **16** by this method because aryl ester hydrolysis is faster than debromination. Acetylation of 8-Br*dhq* (**13**) with Ac_2O/

pyridine gave 8-bromotetra- (**14**) and pentaacetate in the molar ratio 1:2. ^{1}H and ^{13}C NMR data (Me_2CO-d_6): Tables 1 and 2. The identity of the ^{1}H NMR spectrum of **14** to that of the major monobromination product of **5** (see above) verifies that C-8 is the preferred position of bromine substitution [27].

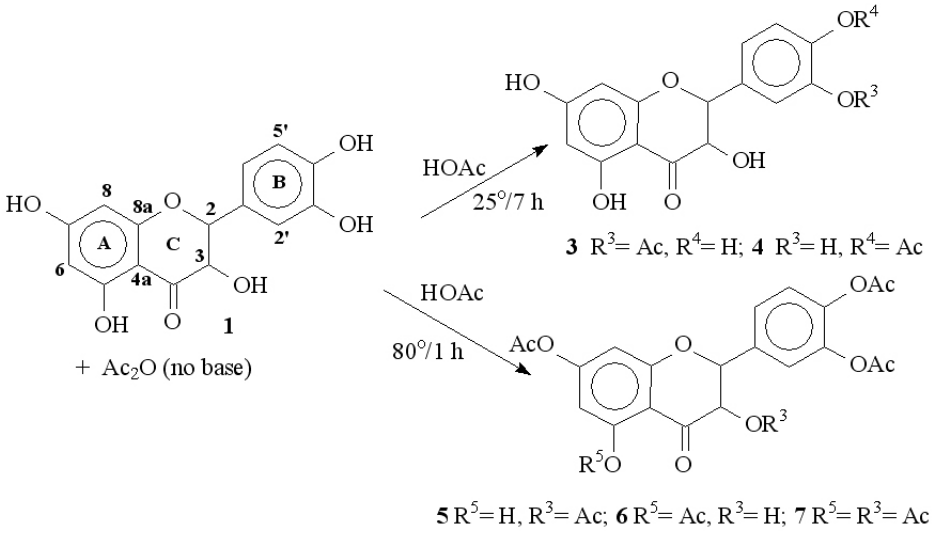
3. Results and Discussion

The extent of *dhq* acetylation by acetic anhydride (Ac_2O) depends sensitively on the reaction conditions [6]. When the reaction is performed at room temperature in the presence of a base catalyst (*e.g.* pyridine or KOAc), the tetraacetate *dhqAc373'4'* (**5**) and the pentaacetate (**7**) are the major products after several hours reaction time whereas *dhq* 7,3',4'-triacetate (**11**) and **5** predominate after a few minutes. Only two monoacetates (*dhqAc3'* (**3**) and *dhqAc4'* (**4**)), three diacetates (*dhqAc3'4'* (**12**), *dhqAc73'* and *dhqAc74'*) and the triacetate **11** are detected after thirty seconds. These observations show that the hydrogen-bonded hydroxy groups (OH-5 and OH-3) have the lowest reactivity, *i.e.*, OH-7 is the preferred acetylation site. The successful synthesis of seven partial acetates on a preparative scale, of the 3-acetate (**2**), *dhqAc33'4'* (**10**) and *dhqAc353'4'* by deacetylation of the pentaacetate (**7**) with sodium sulfite in aqueous methanol, and the identification of twelve more *dhq* acetates formed as minor products of these reactions [6] encouraged us to continue the search for the eight elusive acetylation products.

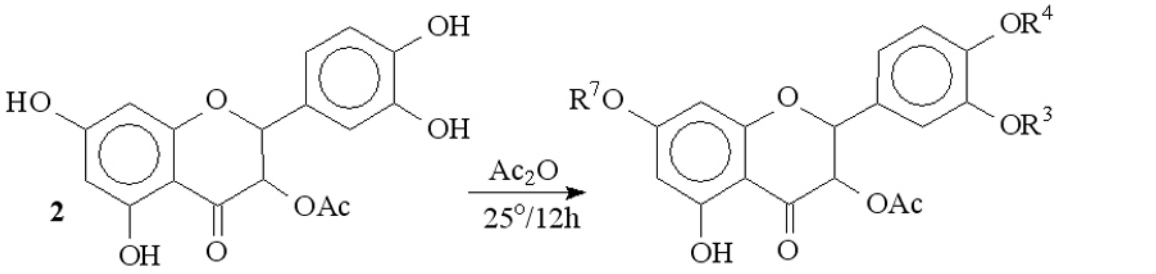
Treatment of *dhq* (**1**) with Ac_2O in the absence of a base catalyst gave (after 7 h at 25° in HOAc) mainly an equimolar mixture of *dhqAc3'* (**3**) and *dhqAc4'* (**4**) [6], two inseparable regioisomers (Scheme 1) that were identified by a NOESY experiment. The addition of a trace of $Cd(NO_3)_2 \cdot 4H_2O$ to the sample solution in acetone-d_6 slows the exchange rate sufficiently to allow the observation of the OH protons as sharp, well-separated singlets [8,9]. Thus cross-peaks at 8.69/7.00 ppm (OH-4'/H-5') and at 8.62/7.19 (OH-3'/H-2') revealed the substrate structures unambiguously; ^{13}C NMR spectra (Table 2) verified these assignments. Only the B-ring carbons were found to resonate at significantly different field strengths, as expected: acetylation of OH-3' (OH-4') shifts the ipso-C signal upfield by 6.3 (6.5) ppm and the *ortho/para* C-13 signals downfield by 6.5-8.1 (7.1-7.9) ppm but has little effect on the chemical shift of C-5' (C-2'/6') [10].

The substrate *dhqAc3* (**2**) was converted primarily to *dhqAc373'4'* (**5**), *dhqAc374'* (**8**), *dhqAc373'* (**9**) and *dhqAc33'4'* (**10**) by excess Ac_2O at ambient temperature (12 h/25°) (Scheme 2) whereas at 80° (again without base catalyst) the reaction yielded penta- and tetraacetates (**7**, **5**), including small amounts of new *dhqAc573'4'* (**6**), and quercetin 3,7,3',4'- tetraacetate.

Partial separation of the major products by PTLC and knowledge of the ^{1}H NMR parameters of *dhq*Ac_5 (**7**) and *dhq*Ac373'4' (**5**) permitted the assignment of the new proton signals at δ 5.33 (d, J = 12.1 Hz), 4.66 (d) and 4.72 ppm (d) to H-2, H-3 and OH-3, respectively, of **6**. The H-2 and H-3 chemical shifts of the new compounds were found to agree with calculated values [6] within 0.03 ppm (Table 1). Small but well-resolved proton signals also revealed the presence of *cis-dhqAc373'4'* (**5c**) and *cis-dhq* pentaacetate (**7c**), *i.e.*, the major products had partially epimerized.

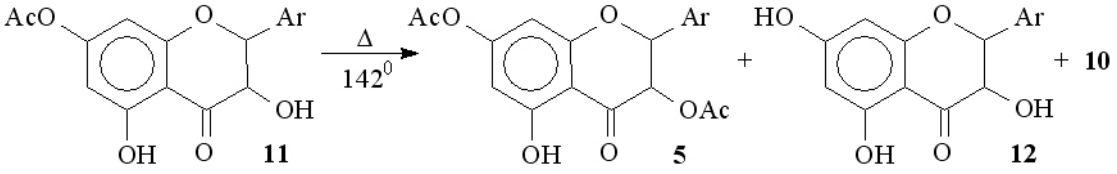


Scheme 1. Acetylation of **1**.



8 $R^7=R^4=$ Ac, $R^3=$H; **9** $R^7=R^3=$ Ac, $R^4=$H; **10** $R^3=R^4=$ Ac, $R^7=$H; **5** $R^7=R^3=R^4=$ Ac

Scheme 2. Acetylation of **2**.



Ar = 3,4-$C_6H_3(OAc)_2$

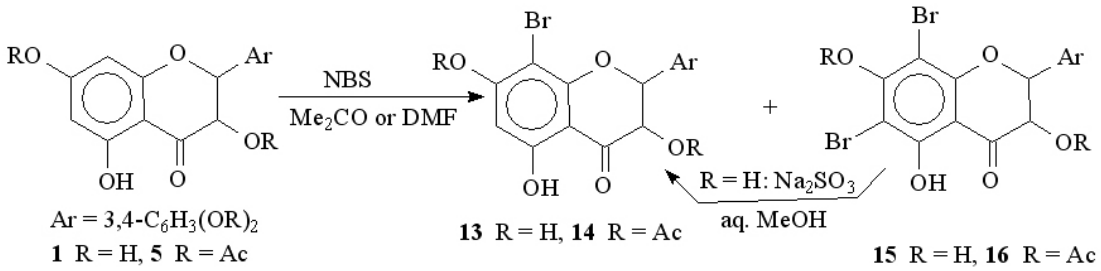
Scheme 3. Transacylation of **11**.

It is well known that in aqueous or alcoholic solution natural *dhq* equilibrates with its more soluble but less stable *cis*-epimer [2,11] which leads ultimately to racemization [12]. The significance of this process for the biosynthesis of proanthocyanidins (condensed tannins) with epicatechin extending units has been pointed out by several researchers [13-15]. Chiroptical and proton NMR studies of the base-catalyzed epimerization of *dhq* 3-*O*-rhamnoside (*astilbin*) led Tominaga and Gaffield [16] to the assignment of absolute configurations to all four *astilbin* diastereomers, including the two 2,3-*cis* isomers (*isoastilbin* and *neoisoastilbin*). Our detection of the 2,3-*cis* epimers of the four *dhq* acetates *dhqAc3* (**2c**), *dhqAc33'4'* (**10c**), *dhqAc373'4'* (**5c**) and *dhq* pentaacetate (**7c**), in addition to nine *cis*-*dhq* methyl ethers previously reported [17], shows that *trans*/*cis* equilibration occurs spontaneously in protic solvents and does not require enzymatic catalysis. However, the low stability of 2,3-*cis*-flavanonols and their derivatives [17] leads to rapid epimerization and has prevented their isolation in pure form. They are readily distinguished from the more abundant *trans* diastereomers by their much smaller H-2/H-3 coupling constants (3 *vs*. 12 Hz). In addition, we found that in all *cis* structures H-2 resonates downfield (Δδ = 0.29-0.31 ppm) and H-3 upfield (Δδ = 0.15-0.21 ppm) from the corresponding peaks of the *trans* isomers.

The appearance of proton signals attributable to unexpected *dhq* acetates with additional acetyl groups in the NMR spectra of several samples of initially pure compounds after long-term storage (up to six months) prompted us to examine their thermal stabilities. Intermolecular acetyl transfer (transacylation) was found to accompany the epimerization observed on heating the partially acetylated compounds *dhqAc73'4'* (**11**) and *dhqAc373'4'* (**5**) above their respective melting points (~150°). *DhqAc373'4'* (**5**) is partially converted to an equimolar mixture of *dhq* pentaacetate (**7**) and *dhqAc33'4'* (**10**), together with some of their respective *cis* isomers, while the 7-*O*-acetyl group of *dhqAc73'4'* (**11**) migrates exclusively to the more nucleophilic oxygen at C-3 giving **5** and the diacetate **12** (Scheme 3). Similar acyl migrations have been reported for a number of flavones [18-20], but the regiochemistry of transacylation of dihydroflavonol esters has not been previously examined.

N-Bromosuccinimide (NBS) (also bromine) is known to oxidize flavanones to flavones, *via* α-bromination followed by dehydrohalogenation with base, and dihydroflavonols to flavonols by the same reaction sequence [21-23]. We have found that in polar solvents (DMF, acetone) excess NBS brominates *dhqAc373'4'* (**5**) rapidly and exclusively in ring A giving 6,8-dibromo*dhqAc373'4'* (**16**) in high yield (Scheme 4).

Traces of 6,8-dibromoquercetin 3,7,3',4'-tetraacetate (**17**) were also recognizable by the low-field ^{1}H NMR peaks of its B-ring protons (H-6' at 8.04, H-2' at 7.99, and H-5' at 7.55 ppm). This result is analogous to the exclusive 6,8-dibromination of the flavanones *naringenin* [24] and *hesperetin* [25] and the preferred ring substitution of methylanisoles [26] by electrophilic aromatic substitution when the reactions are performed in polar media. Under free-radical conditions (1 h reflux in CCl_4 with catalytic amounts of benzoyl peroxide),



Scheme 4. Bromination of *dhq* and *dhqAc373'4'*.

flavones **17** and 8-bromoquercetin 3,7,3',4'-tetraacetate have been reported as major by-products of this reaction [7]. Similarly the photochemical reaction of bromine with *naringenin* triacetate under UV light produces not only the expected 6,8-dibromo derivative but also *apigenin* triacetate [27], presumably by initial C-ring bromination (at C-2 or C-3) and subsequent HBr elimination.

The reaction of *dhqAc373'4'* (**5**) with only one equivalent of NBS is more complex. It usually proceeds beyond the monobromination stage because the initially formed quinoid bromocyclohexadienone ("ketobromide") intermediate [28,29] reacts faster than the starting material which results in the formation of **16** as major product. Approximately one third of the starting material is dibrominated, only one third is converted to 8-bromo*dhqAc373'4'* (**14**) [7] and the rest is recovered unreacted. Attempts to brominate *dhq* pentaacetate (**7**) with NBS failed due to the unfavorable stereoelectronic effect of the bulky acetoxy substituents at C-5 and C-7. However, mono- and dibrominated *dhq* pentaacetate are accessible by *dhq* bromination and subsequent acetylation of the initially formed 8-bromo-*dhq* (**13**) and 6,8-dibromo*dhq* (**15**). With two equivalents of NBS (in acetone) *dhq* is quantitatively converted to **15**. A small excess of NBS (1.1 equivalents) leads to a product mixture containing **13**, its 6-bromo isomer, **15** and *dhq* in the approximate molar ratio 3:3:3:2, *i.e.,* regiospecific monobromination could not be achieved due to the high reactivity of the initially formed ketobromide. But 6,8-dibromo-*dhq* (**15**) can be regioselectively debrominated at C-6 by sodium sulfite in aqueous methanol, analogous to the debromination of 6,8-dibromocatechin [29]. Thus dibromination followed by regioselective monodebromination serves as a successful strategy for the synthesis of monobrominated polyphenols when regioselective monobromination is not feasible. Subsequent acetylation of 8-bromo*dhq* (**13**) produces the same 8-bromo*dhq* tetraacetate **14** (and pentaacetate) formed together with the dibromo*dhq* tetraacetate **16** in the aforementioned reaction of *dhqAc373'4'* (**5**) with one equivalent of NBS [7]. The partial debromination of 6,8-dibromo*dhq* tetraacetate (**16**) with sodium sulfite as alternative route to **14** was not successful because this reaction requires two hydroxy groups in ring A [29]. In that reaction, a mixture of several deacetylation products was formed initially and debrominated subsequently to give *dhq* 3-acetate (**2**) and *alphitonin*, a benzylcoumaranone resulting from C-ring contraction [2].

4. Conclusions

In summary, six new *dhq* acetates were detected among the acetylation products of *dhq* and its 3-acetate (**2**): one tetraacetate (**6**), two triacetates (**8** and **9**), and three 2,3-*cis* stereoisomers (**2c, 5c, 7c**) formed by partial epimerization of the known, more stable *trans* structures. Two *dhq* acetates (**5** and **11**) were found to undergo thermal transesterification by intermolecular acyl migration. Electrophilic substitution by bromine occurs exclusively at C-6 and C-8 of *dhq* and its 3,7,3',4'-tetraacetate, respectively, on treatment with NBS in polar solvents, and dibromination is the main reaction even when equimolar quantities of NBS and substrate are used. 8-Bromo*dhq* (**13**) is readily accessible by regioselective debromination of 6,8-dibromo*dhq* (**15**) with Na_2SO_3 in aqueous MeOH.

Abbreviations

In the abbreviated names, such as *dhqAc373'4'* for dihydroquercetin 3,7,3',4'-tetraacetate (**5**), the numbers conform to standard flavonoid nomenclature (see compound 1 of Scheme 1) and designate the positions of the oxygen atoms of dihydroquercetin (*dhq*) to which the acetyl (*Ac*) groups are attached. Bold compound numbers followed by the letter c (bold) designate 2,3-cis stereoisomers.

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