6-Hydroxychroman-2-carboxylic Acids: Novel Antioxidants[1]

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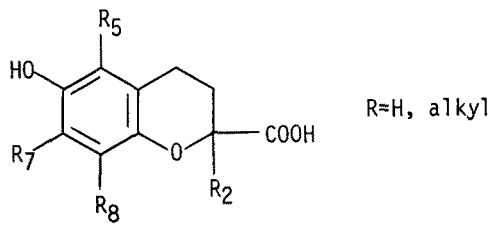
ABSTRACT

6-Hydroxychroman-2-carboxylic acids (I) have been found to be effective antioxidants in animal fats, vegetable oils, and emulsion systems. Two new



R=H, alkyl

I

syntheses of these compounds have been developed. Structure-activity correlations for I with various substituents at C_2, C_5, C_7, and C_8 in various test systems have been obtained. In addition, the homologous chroman acetic acids, which are also antioxidants, and a number of other derived compounds have been synthesized. The most effective antioxidant in this series is the tetramethyl compound I (R_2=R_5=R_7=R_8=CH_3). This compound has activity which compares well with the better commercial antioxidants.

INTRODUCTION

The antioxidant properties of the tocopherols, in particular α-tocopherol [1] and γ-tocopherol [2] have been known (1,2) for many years. The α-tocopherol model, 6-hydroxy-2,2,5,7,8-pentamethylchroman [3], in which the isoprenoid chain of the tocopherol has been replaced by a methyl group also possesses antioxidant activity (3,4). A related compound, 7-tert-butyl-6-hydroxy-2,2,4-trimethylchroman, has been shown (5) to be as effective as butylated hydroxytoluene (BHT) in protecting safflower oil from peroxidation. During the course of some other work, we had the occasion to prepare a number of 6-hydroxychromans. Evaluation of these compounds as antioxidants indicated that several had significant activity. Particularly effective were those 6-hydroxychromans substituted at C_2 with a carboxylic or acetic acid group. In this report, we discuss the synthesis of these acids and related compounds, as well as their antioxidant activity in several test systems.

EXPERIMENTAL PROCEDURES

The synthetic routes employed to obtain the compounds in this study are illustrated by descriptions of the preparations of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [4] and 6-hydroxy-2,5,7,8-tetramethylchroman-2-acetic acid [5]. Methods for the preparation of the other compounds are indicated briefly in "Results and Discussion" and will be supplied in full upon request. All new compounds gave satisfactory microanalyses and had spectra in accord with their structures. Except as noted, all compounds were racemic.

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6-Hydroxy-2,5,7,8-Tetramethylchroman-2-Carboxylic Acid

To a mixture of 152.2 g trimethylhydroquinone in 600 ml methanol and 150 ml trimethyl orthoformate, which was cooled under N_2 in an ice bath, was added 2.5 ml concentrated H_2SO_4 followed dropwise, over 3.0 hr, by 170 ml methyl vinyl ketone. The suspension was stirred for 44 hr without cooling, diluted with ether, washed with H_2O and saturated $NaHCO_3$ solution, and dried (Na_2SO_4). Solvent removal gave 6-hydroxy-2-methoxy-2,5,7,8-tetramethylchroman [8] as 245 g tan solid (melting point [mp] after crystallization from methanol-H_2O 125-126 C).

To a solution of the crude ketal in 1250 ml acetone was added 1000 ml H_2O and 8.30 ml concentrated HCl. The solvent was removed from the solution at reflux until the distilling head temperature reached 92 C. The heat source was removed and the suspension was allowed to cool. At 70 C, 800 ml acetone was added, giving a clear solution. This solution was cooled in an ice bath as crystallization proceeded. The acetone then was removed from the suspension on a rotary evaporator at 30 C. The suspension was cooled again and filtered. The solid was washed with H_2O and dried to give 2,6-dihydroxy-2,5,7,8-tetramethylchroman [10] (6) as a grey-tan powder (mp after crystallization from acetone 128-131 C).

A suspension of the crude hemiketal in 600 ml pyridine was cooled in an ice bath under N_2. Acetic anhydride (900 ml) was added over 3.0 hr. The resulting solution was stirred at 25 C for 18 hr, poured into ice-H_2O, stirred for 2.0 hr, and extracted with benzene and ether. The organic solutions were washed with 2N HCl, brine, saturated $NaHCO_3$ solution and brine, and dried (Na_2SO_4). Solvent removal gave a light yellow oil which was triturated with ether, filtered, and dried to give 4-(2,5-diacetoxy-3,4,6-trimethylphenyl)butan-2-one [13] (7) as 175 g white powder, mp 92.5-93.5 C.

A solution of the purified diacetate in 750 ml dimethylformamide was cooled under N_2 in an ice bath. A solution of 52.7 g potassium cyanide in 110 ml H_2O was added over 10 min. The mixture was stirred with cooling another 10 min and then 122.5 ml 6N H_2SO_4 was added dropwise over 20 min. The viscous mixture (pH 8) was stirred another 30 min with cooling and then brought to pH 4 by the addition of 15 ml 6N H_2SO_4. The mixture was poured into ice-H_2O and extracted with ether. The ether solutions were washed with H_2O and brine and dried (Na_2SO_4). Solvent removal gave 2-cyano-4-(2,5-diacetoxy-3,4,6-trimethylphenyl)butan-2-ol [16] as 267 g viscous resin (mp after crystallization from ether/30-60 C petroleum ether 116.5-118 C).

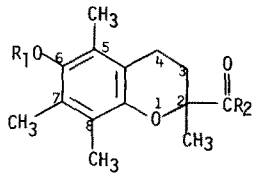
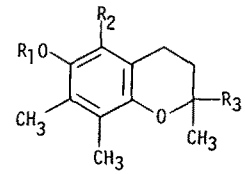
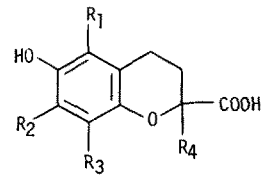
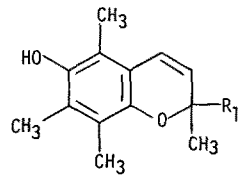
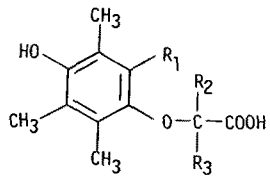
A suspension of the crude cyanohydrin in 1875 ml concentrated HCl was warmed under N_2 to 90 C and kept at this temperature for 40 hr. The suspension was cooled in an ice bath and filtered. The solid was washed with H_2O, air-dried, and then stirred with 2000 ml one-half saturated $NaHCO_3$ solution and 250 ml ether. The aqueous layer was washed with ether, treated with Norite A charcoal, filtered, acidified with 6N HCl, cooled, and filtered. The solid was washed with H_2O and dried to give 165.4 g (66% yield) 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [4] as a white powder, mp 189-191.5 C.

6-Hydroxy-2,5,7,8-Tetramethylchroman-2-Acetic Acid

6-Hydroxy-2-methoxy-2,5,7,8-tetramethylchroman, pre-

TABLE I

Antioxidant Activity of 6-Hydroxychroman-2-Carboxylic Acids and Related Compounds

Compound	Structure	R_1	R_2	R_3	R_4	mp (Solvent)[a]	Antioxidant activity[b]			
							Oxygen analyzer (Percent NDGA[c])	Schaal oven (days)		AOM[c] (hr)
								Soybean oil	Chicken fat	
4		H	OH			190-192 C (H_2O)	92-100	17-36	35	27
28		H	OH (2R)			162-163 C (Et_2O-pet Et_2O)	100	20	35	25
29		H	OH (2S)			161-162.5 C (Et_2O-pet Et_2O)	100	20	35	25
19		CH_3CO	OH			165.5-167 C (Et_2O-pet Et_2O)	0	NR[d]	NR	NR
20		ΦCH_2	OH			154.5-155 C (Et_2O-pet Et_2O)	0	NR	NR	NR
22		H	OCH_3			158.5-161.5 C (Acetone-Et_2O)	151	4	5	7
23		H	OCH_2CH_3			124-126 C (Et_2O)	181	4	5	8
24		H	NH_2			220-220.5 C (Acetone)	106	3	22	5
26		H	CH_3	H		144.5-145.5 C (Et_2O-pet Et_2O)	150	8	9	5
3		H	CH_3	CH_3		93.5-96 C (Et_2O-pet Et_2O)	160	6	5	5
27		H	CH_3	CH_2OH		111-113 C (Et_2O-pet Et_2O)	130	6	8	NR
5		H	CH_3	CH_2COOH		172-173.5 C (EtOH-H_2O)	80	8-12	16	6
21		CH_3CO	CH_3	CH_2COOH		103.5-106 C (Et_2O-pet Et_2O)	0	NR	NR	NR
25		H	CH_3	CH_2COOCH_3		bp 170-173 C/0.2 mm	86	6	10	NR
44		H	H	CH_2COOH		131-132.5 C (benzene)	67	6	17	5
18		H	CH_3	CH_2CH_2COOH (2R)		156.5-158.5 C (EtOAc)	80	6	16	NR
1		H	CH_3	$C_{16}H_{33}$ (α-tocopherol)			7	6	13	5
2		H	H	$C_{16}H_{33}$ (γ-tocopherol)			9	7	29	5
36		CH_3	CH_3	CH_3	CH_2CH_3	210.5-213 C (Acetone-Et_2O)	80	7	6	NR
37		CH_3	CH_3	CH_3	H	208.5-210 C (Acetone-Et_2O)	70	9	12	16
45		H	H	H	CH_3	189.5-192 C (Acetone-Hex)	19	NR	NR	NR
43		H	CH_3	CH_3	CH_3	167.5-168.5 C (Et_2O-pet Et_2O)	47	12	25	7
46		H	$C(CH_3)_3$	H	CH_3	215-220 C (Et_2O-pet Et_2O)	126	5	20	5
47		$CH(CH_3)_2$	$CH(CH_3)_2$	H	CH_3	187.5-190 C (Acetone-Hex)	90	15	25	18
30		COOH				190-192 C (Et_2O-Hex)	92	8	12	NR
31		CH_2COOH				153-155.5 C (EtOH-H_2O)	80	8	16	NR
32		$CH_2COOCH_2CH_3$				113.5-115 C (Acetone-Et_2O)	167	4	5	7
33		H	CH_3	CH_3		109-111.5 C (Et_2O-pet Et_2O)	75	NR	NR	NR
34		CH_3	H	H		205-206.5 C (Acetone-Et_2O)	59	NR	NR	NR
35		CH_3	CH_3	CH_3		157-160 C (Et_2O)	100	3	NR	5
		Control					0	5	5	5

[a]All new compounds gave correct combustion analyses for carbon and hydrogen.

[b]See "Methods" for description of these tests. All compounds were evaluated at 0.02 wt %.

[c]NDGA = nordihydroguaiaretic acid and AOM = active oxygen method.

[d]NR = Not run.

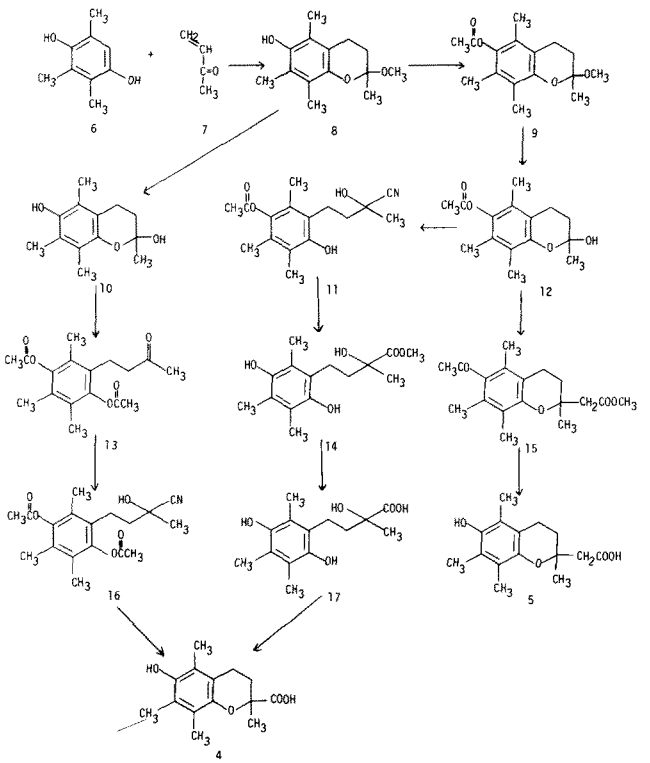


FIG. 1. Syntheses of chroman-2-carboxylic and acetic acids.

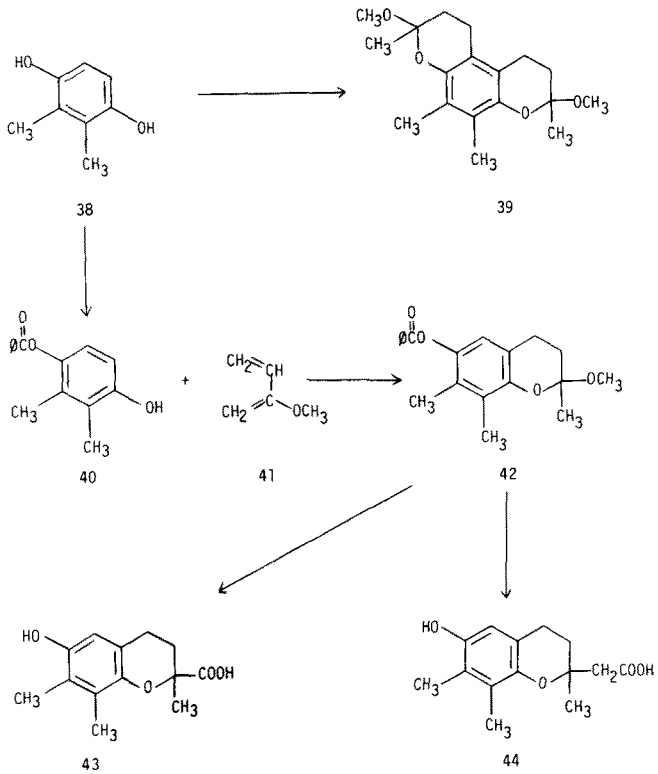


FIG. 2. Alternate syntheses of chroman-2-carboxylic and acetic acids.

pared as described above from 304.4 g trimethylhydroquinone, was acetylated by the method described above to give 6-acetoxy-2-methoxy-2,5,7,8-tetramethylchroman [9] as 570 g red-brown oil (mp 71-72.5 C after evaporative distillation at 175-180 C/0.015 mm). Acid hydrolysis of this compound by the method described above gave 6-acetoxy-2-hydroxy-2,5,7,8-tetramethylchroman [12] as 475 g (90% yield) yellow-tan solid (mp after crystallization from acetone-H_2O 124-126 C).

A suspension of 47.2 g 56% sodium hydride-mineral oil in 1000 ml tetrahydrofuran was stirred, as 209.4 g trimethyl phosphonoacetate was added over 2.25 hr. The white paste was stirred for 15 min, and then a solution of 132.2 g hemiketal [12] in 1000 ml tetrahydrofuran was added over 30 min. The suspension was stirred at 25 C for 18 hr, heated at reflux for 4.0 hr, cooled, diluted with H_2O, and stripped of solvent. The residues from two such reactions were combined and extracted with ether. The ether solutions were washed with H_2O, dried (Na_2SO_4), and stripped of solvent to give methyl 6-acetoxy-2,5,7,8-tetramethylchroman-2-acetate [15] as a red-brown oil (boiling point (bp) of pure material 147-149 C/10 mm). To a solution of this material in 2000 ml ethanol and 2000 ml H_2O, was added 240 g sodium hydroxide. The mixture was stirred under N_2 at 25 C for 4.0 hr, washed with 30-60 C petroleum ether, diluted with H_2O, acidified with concentrated HCl, cooled, and filtered. The solid was washed with H_2O, briefly air-dried, and crystallized from ethanol-H_2O to give 6-hydroxy-2,5,7,8-tetramethylchroman-2-acetic acid [5] as 211 g (80% yield) light tan powder, mp 168-171 C.

Antioxidant Evaluation

The preliminary screening test involved the use of an oxygen analyzer to measure peroxidation by a method developed in these laboratories (8). In this method, hemoglobin is used to peroxidize a 10% safflower oil emulsion, and oxygen removal is measured by an oxygen analyzer. Without antioxidants, all the oxygen is removed in 1.5 min. The results are expressed as percentage of a standard, in this case nordihydroguaiaretic acid (NDGA). The Schaal oven tests (2) were run in 0.2 ml oil or fat in 50 ml beakers at 45 C. The peroxides were titrated daily with KI and $Na_2S_2O_3$ (9). The results are expressed in terms of days to reach 70 meq/kg peroxide in soybean oil and 20 meq/kg peroxide in chicken fat. Active oxygen method (AOM) tests (2) were run at 98 C in soybean oil. The samples were monitored in the same manner as for the Schaal oven tests, and the results are given as hr to reach 70 meq/kg peroxide. In all tests, the antioxidants were added at the legal limit for many food antioxidants of 0.02 wt %.

RESULTS AND DISCUSSION

The compounds we prepared during the course of this study and their antioxidant properties are given in Table I. Our lead substances were 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [4] and 6-hydroxy-2,5,7,8-tetramethylchroman-2-acetic acid [5]. The syntheses of these materials (Fig. 1) illustrate the general approach to most of the substances in the Table. Condensation of trimethylhydroquinone [6] and methyl vinyl ketone [7] in a methanol-trimethyl orthoformate-sulfuric acid mixture gave the ketal [8]. Two routes for the conversion of this material to the acid [4] were developed. In the first of these, ketal [8] was acetylated to the acetate [9] which, upon acid hydrolysis, gave hemiketal [12]. This hemiketal exists to some extent in the open, phenolic ketone form. Thus, treatment of the material with cyanide gave the cyanohydrin [11]. Reaction of this compound with anhydrous hydrogen chloride in methanol followed by hydrolysis of the intermediate iminium salt (10) gave the hydroxyester [14]. Saponification (to [17]) and acid-catalyzed cyclization then gave the desired chromancarboxylic acid [4].

The second route to acid [4] involved initial acid hydrolysis of ketal [8] (to [10]) followed by acetylation to give the diacetate [13]. This material was converted to cyanohydrin [16]. Warming of this cyanohydrin in concentrated hydrochloric acid caused acetate and nitrile hydrolysis and ring closure to give [4] in 66% overall yield.

The hemiketal [12] was also an intermediate in the synthesis of the homologous acid [5]. Horner reaction of this material with trimethyl phosphonoacetate gave an $\alpha\beta$-unsaturated ester which cyclized under the reaction conditions to give the diester [15]. Saponification then gave the chromanacetic acid [5].

The testing results for compounds [4] and [5] indicate that the chromancarboxylic acid [4] is significantly more effective as an antioxidant than the chromanacetic acid [5]. It is thus not surprising that the chroman propionic acid [18] (7) was somewhat less active than [5]. Our initial efforts to determine a structure-activity relationship showed that both the phenolic and carboxylic acid groups were required for maximal activity. Esterification or etherification of the phenol gave compounds [19]-[21] devoid of activity. Esters or amides of the carboxylic acid (compounds [22]-[25]) were at least as good as the parent substance in the oxygen analyzer emulsion system but were much less effective in the other tests. The same statement applies to substances in which the carboxyl was replaced by hydrogen, methyl, or hydroxymethyl (compounds [26], [3], and [27], respectively). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [4] was resolved as its α-methylbenzylamine salt. The enantiomers [28] and [29] were both as effective as [4] antioxidants, a finding in accord with a similar observation (11) concerning d- and dl-α-tocopherol.

The necessity of having the chroman nucleus was ascertained by preparation of compounds [30]-[35]. The chromenes [30]-[32] were obtained by oxidation of the corresponding 6-acetoxychroman acid esters with dichlorodicyanoquinone (DDQ) in toluene (12) followed by saponification and (for [32]) reesterification. Durohydroquinone was benzylated selectively to 4-benzyloxy-2,3,5,6-tetramethylphenol. Reaction of this material with ethyl bromoacetate and ethyl α-bromoisobutyrate (13), followed by saponification and debenzylation, gave compounds [34] and [35]. Reaction of trimethylhydroquinone with chloretone (14) gave acid [33]. None of these compounds was as active as the chroman acid [4].

At this stage, the only uncertainty concerning structure-activity in this series of compounds was the nature of the substituents at C_2, C_5, C_7, and C_8 of the chroman ring. Carrying out the reaction sequence in Figure 1 with ethyl vinyl ketone and acrolein in place of methyl vinyl ketone gave compounds [36] and [37]. Neither was as effective an antioxidant as compound [4]. We then turned our attention to modification of the substituents at C_5, C_7, and C_8. We chose first to prepare less-substituted analogues, since γ-tocopherol [2] is known (11) to be a more effective antioxidant than α-tocopherol [1]. To prepare the γ-analogues, i.e. lacking the methyl at C_5, of acids [4] and [5], we had to devise an additional synthesis (Fig. 2). 2,3-Dimethylhydroquinone [38] reacted with methyl vinyl ketone to give a diadduct [39], rather than the desired monoadduct. The hydroquinone, thus, was converted to the known (15) monobenzoate [40]. This compound reacted only slowly with methyl vinyl ketone. However, heating [40] with 2-methoxybutadiene in a sealed tube (16) cleanly gave ketal [42]. By the methods described previously, ketal [42] was converted to the acids [43] and [44]. In a similar manner, we prepared compound [45] from 4-benzyloxyphenol. Evaluation of these compounds indicated that γ-tocopherol is an anomolous substance and that the usual rule that additional substitution ortho to the phenolic group leads to enhanced activity (2,8) holds for our compounds. We, thus, prepared, by similar means, the 7-tert-butyl and 5,7-diisopropyl compounds [46] and [47]. Neither of these compounds was as effective as [4] in all tests.

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [4] has proven to be the most active compound we have prepared. It is unusual, because it is effective in both animal fats and vegetable oils, in contrast to the tocopherols which have poor activity in the prevention of peroxidation of vegetable oils. Compound [4] has been subjected to extensive secondary testing concerning antioxidant activity in other systems, synergistic effects, stability in oils and fats, and possible modes of action. The results of these tests form the subject of a forthcoming publication.

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