Biogenetically Modeled Syntheses of Heptaacetate Metabolites. Alternariol and Lichexanthone

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Abstract: Methyl 7-(2,4-dimethoxy-6-methylphenyl)-3,5,7-trioxoheptanoate (**8**) was prepared and found to cyclize in aqueous KOH by a Claisen pathway to give benzophenone **29.** The corresponding enol lactone **31** isomerized to **29** in the presence of LiH. Methanolic KOH converted **29** to xanthone **30,** methylation of which gave lichexanthone (**2**). The 2,4-dihydroxy-6-methyl (**25**) and 2-hydroxy-4-methoxy-6-methyl (**17**) analogues of **8** were prepared but cyclized spontaneously to hemiketals **26** and **18.** Nevertheless, by using the hemiketals aldol cyclization followed by lactonization was achieved in the presence of NaOAc–HOAc in the first case and NaOAc alone in the second to give alternariol (**3**) and its methyl ether **32,** respectively. Mechanisms are considered. The relationship of these reactions to the biosynthesis of lichexanthone, alternariol, and related natural products is discussed.

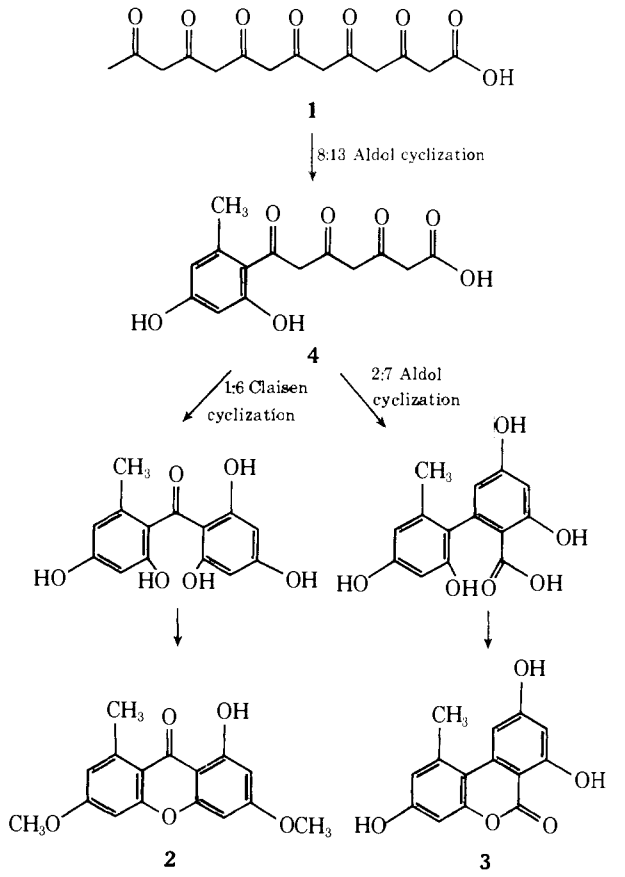
The polyketide group of aromatic natural products comprises many compounds ranging from simple monocyclic ones such as *m*-cresol and orcinol which are formed from a triketo acid to complex ones including tetracycline and daunomycin which require biosynthetic intermediates containing as many as ten carbonyl groups.[1] For some time we have been involved in a program to synthesize various polyketide metabolites along biogenetic lines by controlled cyclizations of appropriate polycarbonyl compounds.[2] Outstanding results have been obtained in the preparation of monocyclic compounds from triketo acids but substantial problems are associated with synthesis of higher polycarbonyl compounds and with the control of their cyclizations to form polycyclic structures.

Among the natural products derived from seven acetate units via enzyme-bound derivatives of 3,5,7,9,11,13-hexaoxotetradecanoic acid (**1**) are two groups of polycyclic compounds for which the synthetic problems would be at a minimum. The first group of metabolites arises from **1** by an aldol cyclization between positions 8 and 13 and a Claisen cyclization between positions 1 and 6. The second group is formed by two aldol cyclizations, one between positions 8 and 13 and the other between 2 and 7 (see Scheme I). Lichexanthone (**2**) is an example of the first group[3,4] and alternariol (**3**) an example of the second.[5,6] These compounds are particularly amenable to synthesis from polycarbonyl compounds because the carbocyclic rings are not fused to each other so that the formation of each ring can be considered as an independent problem.

Details of the biosynthesis of the ring systems are not known for these compounds. In no case is it known which ring forms first, nor even whether the complete polycarbonyl chain is assembled before the first cyclization takes place. It should be noted that both groups of compounds result from an 8:13 aldol cyclization; if this cyclization is the first to occur, triketo acid **4** would be common to both pathways. Via chemical models, we have sought to explore this possibility; the methyl ester of **4** and derivatives having one or both of the phenolic hydroxyl groups methylated have been prepared and the factors controlling their cyclizations investigated.[7]

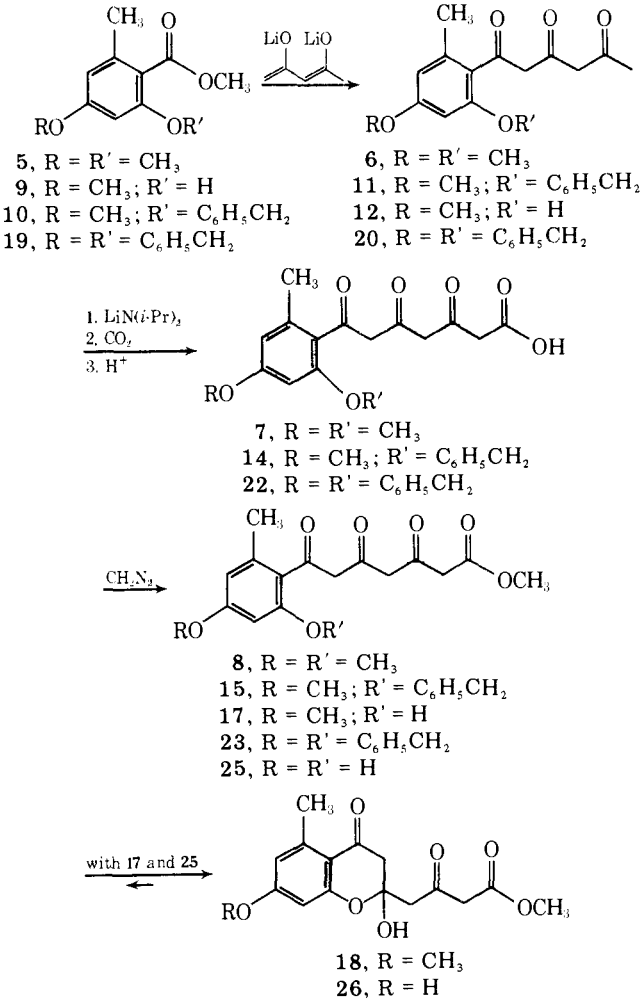
Preparation of Aryl Triketo Esters 8, 17, and 25 (Scheme II). The starting point for syntheses of all three aryl triketo esters was orsellinic acid which had been prepared by carboxylation of the trianion of 2,4,6-heptanetrione, followed by aldol cyclization of the resulting 3,5,7-trioxooctanoic acid.[8] Acylation of dilithioacetylacetone with the dimethyl ether (**5**) of methyl orsellinate gave triketone **6** in 88% yield; the success of this reaction is noteworthy in view of the steric hindrance to attack at the carbonyl group of **5** presented by the two ortho substituents.[9] Carboxylation of the trilithium salt of **6** gave triketo acid **7** (19%), which was esterified by treatment with CH_2N_2 to give 95% of ester **8**.

Scheme I



For the preparation of **17,** the free *o*-hydroxyl group was protected temporarily by benzylation. Ester **10** was prepared from orsellinic acid by treatment with excess CH_2N_2 to give 4-O-methylated ester **9** which was then benzylated (88%) with benzyl chloride and K_2CO_3. Acylation of dilithioacetylacetone again went well in spite of crowding around the ester group of **10;** triketone **11** was obtained in 74% yield. The initial plan was to remove the benzyl group prior to carboxylation. Hydrogenolysis of **11** using a Pd–charcoal catalyst proceeded smoothly but triketone **12** (93%) cyclized immediately to hemiketal **13.** Although hemiketal formation is a reversible process, all attempts to carboxylate **13** met with failure using lithium di-

Scheme II



5, R = R′ = CH_3
9, R = CH_3; R′ = H
10, R = CH_3; R′ = $C_6H_5CH_2$
19, R = R′ = $C_6H_5CH_2$

6, R = R′ = CH_3
11, R = CH_3; R′ = $C_6H_5CH_2$
12, R = CH_3; R′ = H
20, R = R′ = $C_6H_5CH_2$

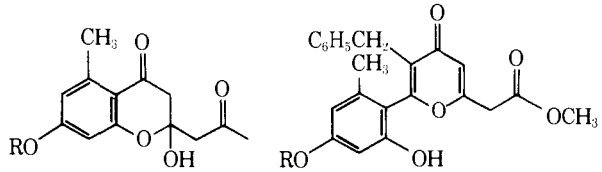
7, R = R′ = CH_3
14, R = CH_3; R′ = $C_6H_5CH_2$
22, R = R′ = $C_6H_5CH_2$

8, R = R′ = CH_3
15, R = CH_3; R′ = $C_6H_5CH_2$
17, R = CH_3; R′ = H
23, R = R′ = $C_6H_5CH_2$
25, R = R′ = H

with 17 and 25

18, R = CH_3
26, R = H

isopropylamide as the ionizing base. The alternative route, carboxylation prior to debenzylation, gave satisfactory results. Treatment of **11** with excess lithium diisopropylamide followed by CO_2 gave unstable triketo acid **14** which was immediately esterified with CH_2N_2 to avoid decarboxylation; ester **15** was obtained in 28% yield. An examination of the remainder of the reaction mixture by chromatography revealed an anomalous byproduct, pyrone ester **16** (9%), in which the benzyl group had undergone a migration.[10] Hydrogenolysis of **15** gave triketo ester **17** (86%) which cyclized immediately to hemiketal **18.** No trace of **17** in equilibrium with **18** could be detected by NMR or other spectral methods but the melting point of **18** was broad, possibly because of equilibration of the two species at the higher temperature.



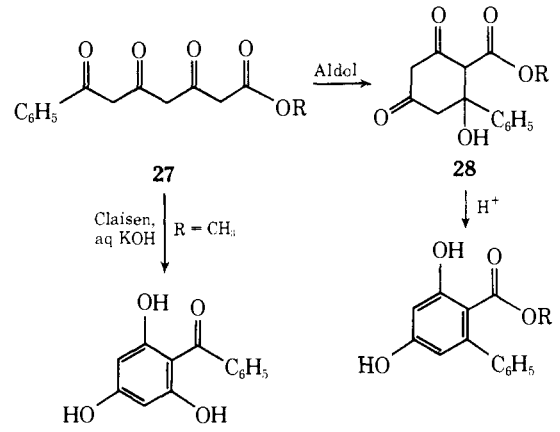
13, R = CH_3
21, R = $CH_2C_6H_5$

16, R = CH_3
24, R = $CH_2C_6H_5$

Unprotected triketo ester **25** was prepared from the dibenzyl ether (**19**) of methyl orsellinate. Acylation of dilithioacetylacetone by **19** was slow at 25 °C but gave 71% of triketone **20.** At 40–50 °C, a debenzylation occurred to give hemiketal **21** (16%) at the expense of **20.**[10] Triketone **20** was carboxylated via the trilithium salt and the resulting acid **22** was immediately esterified with CH_2N_2 to avoid decarboxylation. The two steps gave 15% of ester **23** along with 8% of the benzyl migration product **24.**[10] Hydrogenolysis of **23** gave 70% of hemiketal **26** by way of triketo ester **25.**

Cyclization Reactions. Prior studies[11] of simple triketo acids including the unsubstituted 7-phenyl-3,5,7-trioxoheptanoic acid (**27,** R = H) have shown that aldol cyclization predominates under basic, neutral, and mildly acidic conditions (Scheme III). With the analogous esters, aldol cyclization is

Scheme III



27 **28**

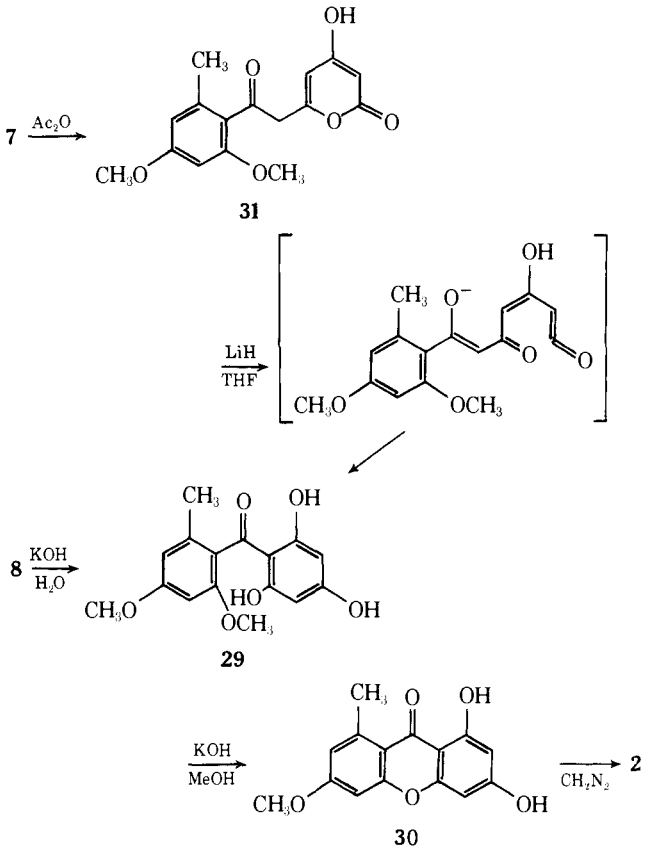
also the major cyclization pathway under conditions ranging from pH 5 to methanolic KOH. The aldol cyclization products (**28**) are stable in base but dehydrate readily under acidic conditions to give resorcinol derivatives. In aqueous KOH, triketo esters (e.g., **27,** R = CH_3) undergo Claisen cyclization to form acylphloroglucinols; the basis for this change of pathway is not understood. Claisen cyclization is not observed with the triketo acids because the carboxyl groups become ionized in base.

Cyclization of triketo acid **7** was investigated first with the expectation of obtaining the aldol cyclization reaction. Surprisingly, no cyclization products could be detected under a variety of conditions. In aqueous solution at pH 5 and at neutrality, acid **7** underwent decarboxylation to give triketone **6.** Acid **7** was stable to aqueous KOH and could be recovered unaltered after a 14-day treatment at room temperature. The failure of aldol cyclization to occur is ascribed to steric hindrance to nucleophilic attack at the 7-carbonyl group by the ortho,ortho′ substituents on the aromatic ring.

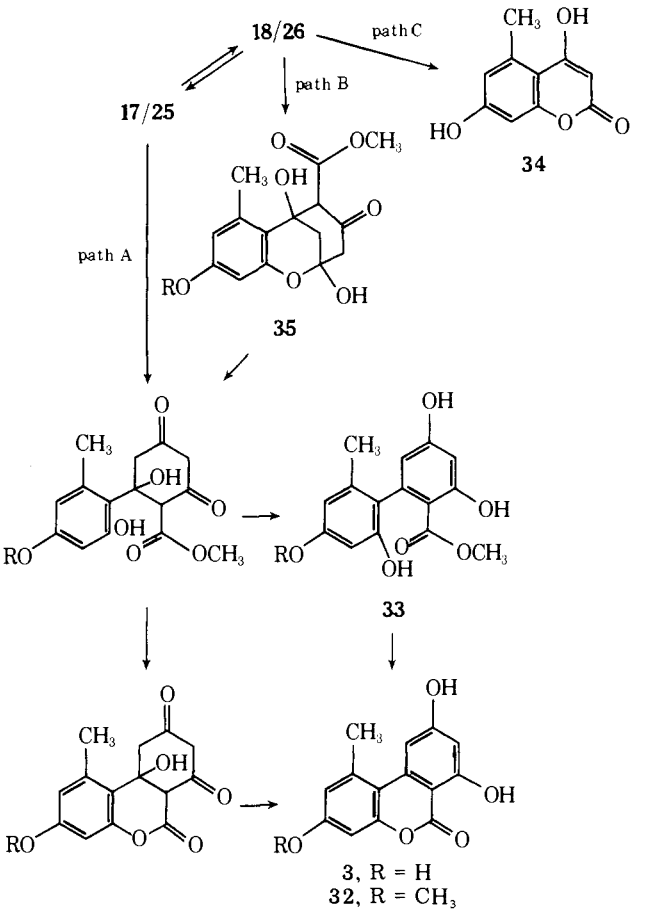
Methyl ester **8** was stable in neutral solution and reacted only slowly in methanolic NaOAc (Scheme IV). The latter gave mixtures of benzophenone **29** derived from Claisen cyclization and chain cleavage products; the cleavage problem was accentuated by increasing the temperature of the reaction. With aqueous KOH at 25 °C for 70 h, 24% of benzophenone **29** was obtained along with an additional 5% of xanthone **30.** Refluxing methanolic KOH gave only xanthone **30** (31%) along with 35% of triketone **6** derived from a cleavage reaction. It was subsequently found that methanolic KOH converts benzophenone **29** to **30** in high yield. Several bases had no permanent effect on ester **8.** These included 1 equiv of LiH in refluxing THF for 12 days, 3 equiv of LiH in refluxing THF for 11 days, and 3 equiv of NaOMe in THF for 3 h at 25 °C. Apparently, these bases formed anionic species that could not cyclize. As expected in the absence of proton sources, cleavage of the triketo ester was not observed.

The final cyclization reaction with the dimethylated compounds involved enol lactone **31** (Scheme IV). This compound was prepared (70% yield) by treatment of acid **7** with acetic anhydride. An earlier study in this laboratory of pyrones of this type led to the discovery of an efficient isomerization to acylphloroglucinols.[12] The reaction is brought about by a variety of bases including nonnucleophilic ones. Acylphloroglucinol

Scheme IV



Scheme V



formation apparently involves an acyl-ketene intermediate; reversion to the triketo ester does not occur. Treatment of **31** with 1 equiv of LiH for 46 h in refluxing THF gave an 87% yield of benzophenone **29.** Lichexanthone (**2**) was prepared from **29** by closure of the heterocyclic ring (87%) using refluxing methanolic KOH followed by methylation (99%) with excess CH_2N_2.[13] The remaining hydroxyl group of **2** is resistant to methylation with this reagent.

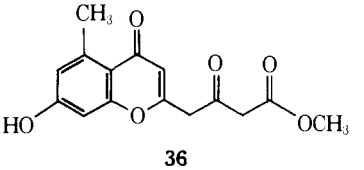
Hemiketals **18** and **26** were studied next (Scheme V). The reversibility of hemiketal formation makes these compounds potential sources of the corresponding triketo esters. Treatment of **18** with methanolic NaOAc gave 64% of the monomethyl ether (**32**) of alternariol; demethylation of **32** with HI gave alternariol (**3**) in 90% yield.[13] In the reaction mixture no biphenic ester (**33,** R = CH_3) or other intermediate between **18** and **32** was detected; neither were the Claisen products, i.e., the benzophenone or xanthone, detected. Under the same conditions, **26** mainly underwent cleavage to give coumarin (**34**) in 62% yield. Other cleavage products were present but were not characterized; very little, if any, alternariol (**3**) was present in the reaction mixture. With less basic catalysts, **3** became a major product at the expense of the cleavage products. With a 4:1 mixture of NaOAc and HOAc **3** and **34** were isolated in yields of 31 and 50%; with equimolar quantities of the catalysts, the respective yields of **3** and **34** became 52 and 47%. Even better yields of **3** could probably have been obtained under more acidic conditions but the reaction would have been too slow to have been useful.

The simplest view of the reactions forming **32** and **3** is that the hemiketals open under the reaction conditions re-forming triketo esters **17** and **25** (path A). The latter compounds would undergo aldol condensation, having less steric hindrance to nucleophilic attack at the 7-carbonyl group than has *o*-methoxy analogue **8.** It is possible that the *o*-hydroxyl groups of **17** and **25** would actually facilitate aldol cyclization by holding the 7-carbonyl group in the plane of the aromatic ring via hydrogen bonding. This conformation would minimize steric hindrance to attack by enolate anion.

An alternative course for these reactions of **18** and **26** would be aldol cyclization *before* cleavage of the hemiketal. An examination of molecular models of **18** and **26** reveals that the carbonyl group adjacent to the aromatic ring is nearly coplanar with it and can be approached readily by the enolate nucleophile to give a strain-free aldol adduct **35.** This pathway (B) would account for the propensity of **18** and **26** to give aldol rather than Claisen cyclization products.

The facile cleavage of **26** but not of **18** to give the corresponding coumarin is consistent with either mechanism. Presumably, coumarin (**34**) is formed directly from **26** (path C) rather than by opening of **26** to triketo ester **25,** cleavage, and recyclization. The free phenolic hydroxyl group of hemiketal **26** is sufficiently acidic to become ionized by NaOAc. Cleavage of the hemiketal ring by base would be more difficult with the anion of **26** than with neutral **18.** Likewise, direct aldol cyclization would be impeded with the anion of **26** because the chromanone carbonyl group would participate in delocalization of the negative charge of the phenoxide ion.

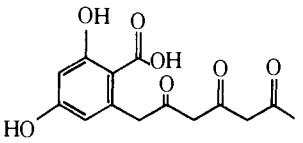
As a final point, the possibility was investigated that hemiketal **26** could be converted to the corresponding xanthone by dehydration to chromone **36** followed by cyclization. Treatment of **26** with *p*-toluenesulfonic acid gave **36** in excellent



yield but subsequent treatment with NaOMe failed to bring about the Claisen cyclization reaction. Cleavage of the chain occurred instead, leading to a 2-methylchromone. It might be

possible to effect xanthone formation by using other conditions, particularly with the methyl ether of **36.** This approach was abandoned, however, because of little apparent relationship to biosynthetic processes; benzophenones are believed to be the precursors of polyketide xanthones.

The proposal for biosynthesis of alternariol (**3**) by the pathway shown in Scheme I[14] is supported by the model studies carried out with **18** and **26** which show that sequential formation of the second aromatic ring and the lactone ring is feasible nonenzymatically. The alternate sequence of cyclizations in which 2:7 aldol cyclization of **1** occurs first is less attractive because **37,** which would arise from the ring closure,



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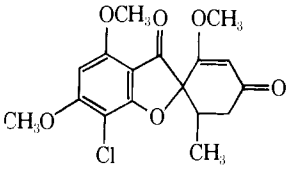
lacks adequate activation for the second cyclization to be a facile process.

The lichexanthone model studies are of interest in relation to the biosynthesis of griseofulvin (**38**).[15-17] In spite of the fact



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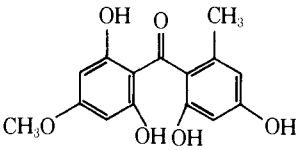

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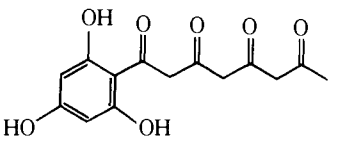

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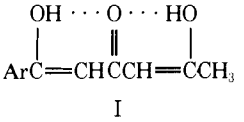
that we have not been able to find conditions under which orcinyl triketo esters will undergo Claisen cyclizations unless the *o*-hydroxyl group has been masked, benzophenone **39,** which lacks protection of the orcinyl *o*-hydroxyl group, is incorporated intact into griseofulvin by *Penicillium griseofulvum*[17] and presumably is a normal intermediate in the biosynthetic pathway. Moreover, *o,o'*-dihydroxybenzophenones are probably precursors of lichexanthone and related[4,18] xanthones. Several possible explanations can be put forward for the apparent discrepancy between the models and the biological systems. First, enzyme-catalyzed reactions sometimes show regiospecificity that cannot be duplicated with simple catalysts; formation of benzophenones from **4** may represent an example of this. Another possibility is that the *o*-hydroxyl group of **4** may become masked prior to cyclization although the protected benzophenones which would result have never been detected. Yet another possibility is that benzophenones may not arise from derivatives of **4** at all; the phloroglucinol ring might be formed first giving tetraketone **40** as an intermediate.

Experimental Section

All melting points were taken with open capillaries unless otherwise noted. Tetrahydrofuran (THF) was freshly distilled from $LiAlH_4$ under N_2. Reactions were routinely monitored by TLC of small aliquots. Organic extracts were dried over $MgSO_4$ before evaporation. Preparative chromatography was carried out on Mallinckrodt CC-4 or similar acid-washed silica gel having low iron content. Elution was effected using hexane containing increasing amounts of Et_2O. Ultraviolet spectra were recorded with EtOH solutions unless otherwise noted. NMR spectra were obtained with a Varian A-60 spectrometer employing tetramethylsilane as the internal standard. Chemical shifts are expressed in δ units. In most cases the OH signals are not reported; many of the OH signals were too broad to be identified with certainty or could not be assigned unambiguously. Low-resolution mass spectra were obtained by direct insertion with an LKB-9000 spectrometer at 70 eV. The parent ion, if any, and three to five of the most intense ions are listed. The high-resolution measurement of **32** was performed in the Mass Spectrometry Laboratory at Battelle Memorial Institute, Columbus, Ohio. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

Methyl *O,O'*-Dimethylorsellinate (5). Orsellinic acid, mp 172–174 °C (lit.[19] mp 176 °C for monohydrate), was prepared in 48% yield by carboxylation of the trilithium salt of 2,4,6-heptanetrione followed by treatment of the resulting triketo acid with pH 5 NaOAc buffer.[8] Treatment of orsellinic acid with 3 equiv of CH_2N_2 for 16 h in Et_2O at 5 °C gave 76% of methyl 4-*O*-methylorsellinate (**9**), mp 60–62 °C (lit.[20] mp 63–65 °C), and 8% of methyl orsellinate, mp 139–140.5 °C (lit.[20] mp 138 °C). Treatment of **9** with excess Me_2SO_4 and K_2CO_3 in refluxing acetone gave 97% of methyl *O,O'*-dimethylorsellinate (**5**), mp 36–38 °C (lit.[20] mp 42–43.5 °C).

1-(2,4-Dimethoxy-6-methylphenyl)-1,3,5-hexanetrione (6). Dilithioacetylacetone (41.7 mmol) was prepared by addition of 4.17 g (41.7 mmol) of acetylacetone to 83.4 mmol of lithium diisopropylamide (prepared from equimolar quantities of *n*-butyllithium and diisopropylamine) in 150 ml of THF at 0 °C under N_2. Ester **5** (2.5 g, 11.9 mmol) was added to the dilithium salt and the mixture was stirred at 25 °C for 16 h. The solvent was evaporated in vacuo and the residue was acidified with cold, dilute HCl and extracted with Et_2O. The extract was dried, evaporated, and chromatographed to give unreacted starting materials, followed by 2.9 g (88%) of triketone **6** as a yellow oil which crystallized on lengthy standing: mp 63–65 °C after recrystallization from hexane; IR (neat) 1725–1550 cm^{-1}; UV 262 nm (ϵ 11 100), 320 (8900); NMR ($CDCl_3$) (mixture of keto–enol tautomers containing approximately 50% bisenol, 25% of each monoenol, and a trace of the unenolized form) 2.72 and 2.80 (aryl CH_3's), 3.75 (OCH_3's) and 6.33 (aromatic CH's) for all tautomers. Remaining singlet signals assigned to individual tautomers:



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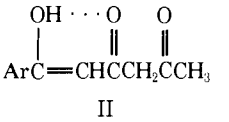

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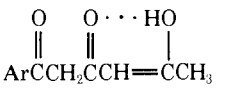

III

(I) 1.95 (CH_3), 5.61 (4-CH), 5.80 (2-CH); (II) 2.24 (CH_3), 3.41 (4-CH_2), 5.32 (2-CH); (III) 2.00 (CH_3), 3.72 (2-CH_2), 5.24 (4-CH); [ArC(=O)CH_2C(=O)CH_2C(=O)CH_3] 2.18 (CH_3), 3.51 (4-CH_2), 3.95 (2-CH_2). Anal. Calcd for $C_{15}H_{18}O_5$: C, 64.75; H, 6.47. Found: C, 64.74; H, 6.58.

7-(2,4-Dimethoxy-6-methylphenyl)-3,5,7-trioxoheptanoic Acid (7). Triketone **6** (1.0 g, 3.6 mmol) in THF (10 ml) was added to lithium diisopropylamide (14.4 mmol) in THF (50 ml) at 0 °C under N_2. After 10 min, CO_2 was bubbled into the red-brown reaction mixture for 5 min. The solvent was evaporated in vacuo and the residue was acidified with cold, dilute HCl and extracted with $CHCl_3$. The $CHCl_3$ solution was extracted with 5% aqueous $NaHCO_3$ and the latter was acidified and reextracted with $CHCl_3$. The organic extract was dried, evaporated, and chromatographed. The crude product was crystallized from Et_2O–hexane and recrystallized from $CHCl_3$–hexane to give 0.218 g (19%) of **7:** mp 84–85.5 °C; IR (KBr) 1720, 1620–1575 cm^{-1}; UV 276 nm (ϵ 16 800), 330 (7600); NMR ($CDCl_3$–CD_3SOCD_3) mono- and bisenol tautomers involving the 4 and 6 positions. Anal. Calcd for $C_{16}H_{18}O_7$: C, 59.62; H, 5.63. Found: C, 59.41; H, 5.81.

Methyl 7-(2,4-Dimethoxy-6-methylphenyl)-3,5,7-trioxoheptanoate (8). Triketo acid **7** (2.65 g, 8.23 mmol) in 50 ml of Et_2O was treated with 7.8 mmol of CH_2N_2 for 30 s. The ethereal solution was washed with aqueous $NaHCO_3$, dried, and evaporated to give 2.5 g (95%) of ester **8** as a yellow oil: IR (neat) 1740, 1640–1560 cm^{-1}; UV 230 nm (ϵ 8100), 303 (9700), 334 (sh, 8400); NMR ($CDCl_3$) showed a mixture containing mainly the mono- and bisenol tautomers involving the 4 and 6 positions. An analytical sample was prepared by chromatography. Anal. Calcd for $C_{17}H_{20}O_7$: C, 60.71; H, 5.99. Found: C, 60.59; H, 6.03.

6-(2,4-Dimethoxy-6-methylphenacyl)-4-hydroxy-(2*H*)-pyran-2-one (31). Crude triketo acid **7**, obtained from carboxylation of the trilithium salt of 1.0 g (3.6 mmol) of triketone **6**, was treated with Ac_2O (20 ml) for 26 h at 5 °C. The yellow precipitate was separated by filtration and washed with cold Et_2O to give 0.771 g (70%) of enol lactone **31**: mp 182.5–184 °C after recrystallization from CH_3CN; IR (KBr) 1680, 1650, 1600, 1580 cm^{-1}; UV 230 nm (sh, ϵ 10 800), 280 (10 800); NMR ($CDCl_3$–CD_3SOCD_3) 2.10 (s, 3, CH_3), 3.81 (s, 3, OCH_3), 3.92 (s, 2, CH_2), 5.31 (d, 1, 3-CH, J = 2.0 Hz), 5.98 (d, 1, 5-CH, J = 2.0 Hz), 6.38 (s, 2, aromatic); MS *m/e* (rel intensity) 304 (parent, 6), 273 (26), 179 (100), 152 (46). Anal. Calcd for $C_{16}H_{16}O_6$: C, 63.15; H, 5.30. Found: C, 62.94; H, 5.27.

Methyl 2-*O*-Benzyl-4-*O*-methylorsellinate (10). A mixture of ester **9** (4.5 g, 23 mmol), benzyl chloride (3.2 g, 25.2 mmol), K_2CO_3 (3.3 g, 25.2 mmol), and hexamethylphosphoramide (40 ml) was heated at 135 °C for 1.5 h, cooled, diluted threefold with H_2O, and extracted with Et_2O. The extract was washed with H_2O, dried, evaporated in vacuo, and chromatographed to give 5.8 g (88%) of ester **10** as a pale yellow oil: IR (neat) 1720, 1600 cm^{-1}; UV 221 nm (ϵ 13 900), 248 (5100), 283 (2500); NMR ($CDCl_3$) 2.29 (s, 3, CH_3), 3.69 (s, 3, OCH_3), 3.82 (s, 3, OCH_3), 5.01 (s, 2, CH_2), 6.32 (s, 2, aromatic), 7.31 (s, 5, C_6H_5). Anal. Calcd for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34. Found: C, 71.13; H, 6.44.

1-(2-Benzyloxy-4-methoxy-6-methylphenyl)-1,3,5-hexanetrione (11). Dilithioacetylacetone [48.4 mmol, prepared from 4.84 g (48.4 mmol) of acetylacetone and 96.8 mmol of lithium diisopropylamide in 170 ml of THF at 0 °C under N_2] was acylated with ester **10** (6.9 g, 24.1 mmol) for 15 h at 25 °C. Work-up by the procedure used with **6** gave 6.35 g (74%) of triketone **11** as yellow crystals: mp 58.5–60.5 °C after recrystallization from hexane; IR (KBr) 1735–1670, 1610–1570 cm^{-1}; UV 281 nm (ϵ 11 000), 322 (sh, 8800); NMR ($CDCl_3$) (mixture of keto–enol tautomers containing approximately 40% of dienol, 40% of 2-enol, and 20% of 4-enol plus a trace of the unenolized tautomer) 2.78 and 2.84 (aryl CH_3's), 3.75 (OCH_3's), 5.04 ($C_6H_5CH_2$'s), 6.37 (aromatic CH's), 7.33 and 7.37 (C_6H_5's) for all tautomers. Remaining singlet signals assigned to individual tautomers: (I) 1.94 (CH_3), 5.42 (4-CH), 5.83 (2-CH); (II) 2.14 (CH_3), 3.35 (4-CH_2), 5.42 (2-CH); (III) 1.97 (CH_3), 3.79 (2-CH_2), 5.19 (4-CH); [ArC(=O)CH_2C(=O)CH_2C(=O)CH_3] 2.10 (CH_3), 3.48 (4-CH_2), 3.98 (2-CH_2). Anal. Calcd for $C_{21}H_{22}O_5$: C, 71.17; H, 6.26. Found: C, 70.93; H, 6.22.

2-Acetonyl-2-hydroxy-7-methoxy-5-methylchroman-4-one (13). A mixture of triketone **11** (0.500 g, 1.41 mmol) and 10% Pd–charcoal (0.2 g) in 25 ml of EtOH was treated with H_2 at 25 °C and atmospheric pressure to give hemiketal **13** (0.348 g, 93%) as an oil which crystallized on standing: mp 116.5–117.5 °C after recrystallization from $CHCl_3$–hexane; IR (KBr) 1730, 1640, 1610, 1570 cm^{-1}; UV 228 nm (ϵ 13 800), 275 (15 200), 308 (5500); NMR ($CDCl_3$) 2.32 (s, 3, CH_3), 2.62 (s, 3, CH_3), 2.69 (d, 1, CH_2, J = 17 Hz), 2.78 (m, 2, CH_2), 3.26 (d, 1, CH_2, J = 17 Hz), 3.80 (s, 3, OCH_3), 6.00 (s, 1, OH), 6.29 (d, 1, aromatic, J = 2.5 Hz), 6.40 (d, 1, aromatic, J = 2.5 Hz); MS *m/e* (rel intensity) 264 (parent, 12), 207 (25), 206 (45), 165 (100), 164 (88), 136 (31). Triketone **12** was not detected. Anal. Calcd for $C_{14}H_{16}O_5$: C, 63.63; H, 6.10. Found: C, 63.63; H, 6.16.

Attempted Carboxylation of 13. Chromone **13** (0.132 g, 0.5 mmol) was treated with 2.5 mmol of lithium diisopropylamide in 45 ml of THF for 10 min at 0 °C under N_2 followed by CO_2. The usual work-up gave only unaltered **13**.

Methyl 7-(2-Benzyloxy-4-methoxy-6-methylphenyl)-3,5,7-trioxoheptanoate (15). Triketone **11** (4.0 g, 11.3 mmol) was treated with lithium diisopropylamide (45.2 mmol) in 180 ml of THF for 15 min at 0 °C followed by gaseous CO_2. After evaporation of the solvent in vacuo, the residue was acidified with cold, dilute HCl and extracted with Et_2O. The ethereal solution was extracted with aqueous $NaHCO_3$ and the aqueous extract was acidified and reextracted with Et_2O. The ethereal solution was dried and evaporated in vacuo to give 2.3 g of crude triketo acid **14** as an oil which was immediately treated for 30 s with an equivalent amount of ethereal CH_2N_2. The solution was treated with a small amount of HOAc to remove excess CH_2N_2, washed with H_2O and with aqueous $NaHCO_3$, dried, evaporated in vacuo, and chromatographed to give two product fractions. The first fraction (0.423 g, 9%) was identified as methyl 5-benzyl-6-(2-hydroxy-4-methoxy-6-methylphenyl)-4-oxo-(4*H*)-pyran-2-acetate (**16**): yellow crystals with mp 125.5–127 °C after recrystallization from $CHCl_3$–hexane; IR (KBr) 1755, 1625–1590 cm^{-1}; UV 278 nm (ϵ 18 400), 308 (31 900); NMR ($CDCl_3$) 2.50 (s, 3, CH_3), 3.29 (s, 2, CH_2), 3.63 (s, 3, OCH_3), 3.84 (s, 3, OCH_3), 3.97 (s, 2, CH_2), 5.61 (s, 1, vinyl), 6.27 (d, 1, aromatic, J = 2.5 Hz), 6.92 (d, 1, aromatic, J = 2.5 Hz), 7.57 (m, 5, C_6H_5); MS *m/e* (rel intensity) 394 (parent, 86), 252 (93), 251 (100), 143 (62), 101 (40). Anal. Calcd for $C_{23}H_{22}O_6$: C, 70.04; H, 5.62. Found: C, 70.23; H, 5.67.

The second fraction (1.3 g, 28%) was a yellow oil identified as triketo ester **15**: IR (neat) 1760–1720, 1620–1570 cm^{-1}; UV 224 nm (sh, ϵ 12 700), 284 (10 500), 301 (10 200); NMR ($CDCl_3$) showed a mixture of keto–enol tautomers containing mainly the mono- and bisenol tautomers involving the 4 and 6 positions; MS *m/e* (rel intensity) 412 (parent, 2), 297 (6), 255 (22), 165 (15), 91 (100). Anal. Calcd for $C_{23}H_{24}O_7$: C, 66.98; H, 5.87. Found: C, 67.11; H, 5.92.

2-(3-Carbomethoxyacetonyl)-2-hydroxy-7-methoxy-5-methylchroman-4-one (18). A mixture of ester **15** (0.650 g, 1.58 mmol) and 10% Pd–charcoal (0.25 g) in EtOH (20 ml) was treated with H_2 at 25 °C and atmospheric pressure. After uptake of H_2 had ceased, the catalyst was removed by filtration and the solvent was evaporated in vacuo to give 0.437 g (86%) of hemiketal **18** as a yellow oil which crystallized from Et_2O: mp 51–58.5 °C after three recrystallizations from Et_2O (the compound appeared to undergo a change in crystal structure at room temperature); IR (KBr) 1750, 1720, 1675, 1605, 1570 cm^{-1}; UV 228 nm (ϵ 15 700), 275 (16 600), 308 (5900); NMR ($CDCl_3$) 2.54 (s, 3, CH_3), 2.83 (s, 2, CH_2), 2.92 (d, 1, CH_2, J = 16 Hz), 3.31 (d, 1, CH_2, J = 16 Hz), 3.63 (s, 2, CH_2), 3.71 (s, 3, OCH_3), 3.77 (s, 3, OCH_3), 6.26 (d, 1, aromatic, J = 2 Hz), 6.35 (d, 1, aromatic, J = 2 Hz); MS *m/e* (rel intensity) 322 (parent, 5), 207 (40), 206 (40), 165 (100), 164 (75), 136 (25). Anal. Calcd for $C_{16}H_{18}O_7$: C, 59.62; H, 5.63. Found: C, 59.59; H. 5.81.

Methyl *O,O'*-Dibenzylorsellinate (19). Methyl orsellinate, mp 139–140.5 °C (lit.[20] mp 138 °C) after recrystallization from aqueous EtOH, was prepared in 94% yield by treatment of orsellinic acid with 1.1 equiv of ethereal CH_2N_2 for 30 s. A mixture of methyl orsellinate (1.55 g, 8.52 mmol), benzyl chloride (2.70 g, 21.3 mmol), K_2CO_3 (2.93 g, 21.3 mmol), and 40 ml of hexamethylphosphoramide was heated at 135 °C for 1.5 h, cooled, added to 200 ml of H_2O, and extracted with Et_2O. The extract was washed with H_2O, dried, evaporated in vacuo, and chromatographed to give 2.65 g (86%) of ester **19** as an oil which crystallized on standing: mp 62–63.5 °C after recrystallization from $CHCl_3$–pentane; IR (KBr) 1710, 1590 cm^{-1}; UV 215 nm (ϵ 26 000), 249 (6600), 280 (3800); NMR ($CDCl_3$) 2.27 (s, 3, CH_3), 3.82 (s, 3, OCH_3), 4.98 (s, 2, CH_2), 5.00 (s, 2, CH_2), 6.40 (s, 2, aromatic). 7.33 (m, 10, C_6H_5). Anal. Calcd for $C_{23}H_{22}O_4$: C, 76.22; H, 6.12. Found: C, 76.21; H, 6.13.

1-(2,4-Dibenzyloxy-6-methylphenyl)-1,3,5-hexanetrione (20). Dilithioacetylacetone [65 mmol, prepared from 6.5 g (65 mmol) of acetylacetone and 130 mmol of lithium diisopropylamide in 330 ml of THF at 0 °C under N_2] was treated with ester **19** (7.85 g, 21.7 mmol) for 36 h at 25 °C. Work-up by the procedure used with **6** gave 6.8 g (71%) of triketone **20** as a yellow oil which crystallized when triturated with Et_2O–pentane: mp 55.5–57 °C after recrystallization from hexane; IR (KBr) 1750–1700, 1640–1540 cm^{-1}; UV 284 nm (ϵ 13 100), 321 (11 100); NMR ($CDCl_3$) (mixture of keto–enol tautomers containing approximately 40% of 2-enol, 30% of 4-enol, and 30% of bisenol plus a trace of the unenolized tautomer) 2.27 and 2.32 (aryl CH_3's), 5.00 ($C_6H_5CH_2$'s), 6.43 (aromatic CH's), 7.30 and 7.34 (C_6H_5's) for all tautomers. Remaining singlet signals assigned to individual tautomers: (II) 2.14 (CH_3), 3.33 (4-CH_2), 5.37 (2-CH); (III) 1.96 (CH_3), 3.77 (2-CH_2), 5.17 (4-CH); (I) 1.92 (CH_3), 5.37 (4-CH), 5.82 (2-CH); [ArC(=O)CH_2C(=O)CH_2C(=O)CH_3] 2.10 (CH_3), 3.47 (4-CH_2), 3.98 (2-CH_2). The analytical sample, dried at 25 °C and 0.5 mm prior to analysis, analyzed as the hemihydrate. Anal. Calcd for $C_{27}H_{26}O_5 \cdot 0.5H_2O$: C, 73.78; H, 6.19. Found: C, 73.54, 73.83; H, 6.08, 6.34.

In another reaction, 37 mmol of dilithioacetylacetone was treated with 6.7 g (18.5 mmol) of ester **19** for 24 h at 40 °C followed by 12 h at 50 °C. Work-up as before gave 3.2 g (39%) of the hemihydrate of **20** plus 1.0 g (16%) of 2-acetonyl-2-hydroxy-7-benzyloxy-5-methylchroman-4-one (**21**): mp 125–126 °C after recrystallization from CH_2Cl_2–hexane; IR (KBr) 1690, 1675, 1610, 1570 cm^{-1}; UV 228 nm (ϵ 14 200), 276 (17 700), 308 (6000); NMR ($CDCl_3$) 2.26 (s, 3, CH_3), 2.59 (s, 3, CH_3), 2.66 (d, 1, CH_2, J = 17 Hz), 2.73 (m, 2, CH_2), 3.32 (d, 1, CH_2, J = 17 Hz), 5.00 (s, 2, $C_6H_5CH_2$), 5.99 (s, 1, OH), 6.33 (d, 1, aromatic, J = 2.5 Hz), 6.43 (d, 1, aromatic, J = 2.5 Hz), 7.34 (s, 5, C_6H_5). Anal. Calcd for $C_{20}H_{20}O_5$: C, 70.59; H, 5.92. Found: C, 70.68; H, 6.08.

Treatment of **21** (0.175 g, 0.51 mmol) with H_2 at atmospheric

pressure and 25 °C in the presence of 10% Pd–charcoal followed by catalytic *p*-toluenesulfonic acid in refluxing benzene (13 h) and by chromatography gave 0.077 g (65%) of 2-acetonyl-7-hydroxy-5-methylchromone: mp 215–217 °C after recrystallization from benzene–ethyl acetate; IR (KBr) 1725, 1650, 1590–1510 cm^{-1}; UV 244 nm (ϵ 16 400), 252 (17 000), 294 (11 400), 332 (sh, 1200); NMR (CD_3COCD_3–CD_3SOCD_3) 2.25 (s, 3, CH_3), 2.71 (s, 3, CH_3), 3.82 (s, 2, CH_2), 6.03 (s, 1, vinyl), 6.64 (s, 2, aromatic). Anal. Calcd for $C_{13}H_{12}O_4$: C, 67.23; H, 5.21. Found: C, 67.34; H, 5.24.

Methyl 7-(2,4-Dibenzyloxy-6-methylphenyl)-3,5,7-trioxoheptanoate (23). Triketone **20** (15.5 g, 36.0 mmol) was treated with lithium diisopropylamide (180 mmol) in 250 ml of THF at 0 °C under N_2 for 15 min followed by gaseous CO_2. Isolation by the procedure used with **15** gave 10.0 g of crude triketo acid **22** which was treated for 30 s with 1 equiv of CH_2N_2 to give two products after chromatography. The first one (1.30 g, 8%) was a yellow oil which crystallized on standing and was identified as methyl 5-benzyl-6-(4-benzyloxy-2-hydroxy-6-methylphenyl)-4-oxo-(4*H*)-pyran-2-acetate (**24**): mp 97–98.5 °C after recrystallization from MeOH; IR (KBr) 1735, 1620–1570 cm^{-1}; UV 228 nm (ϵ 20 400), 305 (29 100); NMR ($CDCl_3$) 2.50 (s, 3, CH_3), 3.25 (s, 2, CH_2), 3.61 (s, 3, OCH_3), 3.92 (s, 2, CH_2), 5.05 (s, 2, CH_2), 5.60 (s, 1, vinyl), 6.71 (d, 1, aromatic, J = 2 Hz), 6.93 (d, 1, aromatic, J = 2 Hz), 7.17–7.72 (m, 10, C_6H_5). Anal. Calcd for $C_{29}H_{26}O_6$: C, 74.03; H, 5.57. Found: C, 74.02; H, 5.80.

The second fraction (2.7 g, 15%) was a yellow oil, identified as triketo ester **23:** IR (neat) 1745, 1670–1570 cm^{-1}; UV 285 nm (ϵ 8900), 337 (6800); NMR ($CDCl_3$) showed a mixture of keto–enol tautomers involving the 4 and 6 positions. The analytical sample was prepared by rechromatography on silica gel. Anal. Calcd for $C_{29}H_{28}O_7$: C, 71.30; H, 5.78. Found: C, 71.07; H, 5.88.

2-(3-Carbomethoxyacetonyl)-2,7-dihydroxy-5-methylchroman-4-one (26). Treatment of ester **23** (2.5 g, 5.12 mmol) and 10% Pd–charcoal (0.3 g) in EtOH (75 ml) with H_2 at 25 °C and atmospheric pressure until uptake of H_2 had ceased gave, after removal of the catalyst by filtration and evaporation of the solvent in vacuo, 1.1 g (70%) of hemiketal **26:** mp 141.5–142 °C after recrystallization from acetone–hexane; IR (KBr) 1735, 1715, 1660, 1610, 1580 cm^{-1}; UV 218 nm (ϵ 15 600), 232 (12 500), 279 (14 400), 308 (sh, 6300); NMR (CD_3SOCD_3) 2.51 (s, 3, CH_3), 2.51 (d, 1, CH_2, J = 16 Hz), 3.03 (d, 1, CH_2, J = 16 Hz), 3.12 (s, 2, CH_2), 3.69 (s, 3, OCH_3), 3.75 (s, 2, CH_2), 6.19 (d, 1, aromatic, J = 2.5 Hz), 6.27 (d, 1, aromatic, J = 2.5 Hz); MS *m/e* (rel intensity) 308 (parent ion, not detected), 290 (8), 192 (68), 151 (33), 150 (100), 122 (38), 74 (70). Anal. Calcd for $C_{15}H_{16}O_7$: C, 58.44; H, 5.23. Found: C, 58.11; H, 5.10.

Treatment of Triketo Ester 8 with Aqueous KOH. Ester **8** (1.04 g, 3.1 mmol) was treated with 0.5 M aqueous KOH (40 ml) for 70 h at 25 °C. The reaction mixture was acidified with cold, dilute HCl and extracted with Et_2O. The extract was dried, evaporated, and chromatographed to give 0.039 g (5%) of 1,3-dihydroxy-6-methoxy-8-methylxanthone (**30**): mp 269–270.5 °C after recrystallization from CH_3OH (lit.[21] mp 260–262 °C); IR (KBr) 1660, 1620, 1570, 1525 cm^{-1}; UV 241 nm (ϵ 40 000), 253 (sh, 21 800), 310 (19 400), 340 (9000); NMR (CD_3SOCD_3) 2.69 (s, 3, CH_3), 3.86 (s, 3, OCH_3), 6.14 (d, 1, aromatic, J = 2.5 Hz), 6.25 (d, 1, aromatic, J = 2.5 Hz), 6.67 (d, 1, aromatic, J = 2.5 Hz), 6.75 (d, 1, aromatic, J = 2.5 Hz), 12.45 (br s, 1, OH), 14.98 (s, 1, OH); MS *m/e* (rel intensity) 272 (100), 243 (10), 229 (10). Anal. Calcd for $C_{15}H_{12}O_5$: C, 66.17; H, 4.44. Found: C, 65.99; H, 4.28.

A second fraction (0.230 g, 24%) was identified as 2,4,6-trihydroxy-2′,4′-dimethoxy-6′-methylbenzophenone (**29**): mp 157.5–159 °C after recrystallization from C_6H_6–hexane; IR (KBr) 1635, 1585 cm^{-1}; UV 228 nm (ϵ 18 400), 300 (21 800), 332 (8700); NMR ($CDCl_3$–CD_3COCD_3) 2.18 (s, 3, CH_3), 3.71 (s, 3, OCH_3), 3.81 (s, 3, OCH_3), 5.92 (s, 2, aromatic), 6.39 (s, 2, aromatic); MS *m/e* (rel intensity) 304 (parent, 12), 289 (18), 274 (13), 273 (67), 272 (15), 152 (100), 123 (16). Anal. Calcd for $C_{16}H_{16}O_6$: C, 63.15; H, 5.30. Found: C, 63.04; H, 5.40.

Treatment of 8 with Methanolic KOH. Ester **8** (1.00 g, 2.97 mmol) was treated with 10% methanolic KOH for 7.5 h at reflux. Evaporation of the solvent followed by the same work-up as above gave 0.289 g (35%) of triketone **6** followed by 0.252 g (31%) of xanthone **30.**

Treatment of 8 with Methanolic NaOAc. Ester **8** (1.27 g, 3.78 mmol) was treated with 1 M methanolic NaOAc (50 ml) for 96 h at 25 °C. Work-up as above gave 0.030 g (4%) of 2,4-dimethoxy-6-methylacetophenone, mp 40–40.5 °C after recrystallization from hexane (lit.[22] mp 41–42 °C), followed by a large amount of unaltered **8** and finally 0.088 g (8%) of benzophenone **29.** Treatment of **8** (0.736 g, 2.19 mmol) with 1 M methanolic NaOAc (25 ml) for 96 h at reflux gave 0.126 g (24%) of 1-(2,4-dimethoxy-6-methylphenyl)-1,3-butanedione, mp 65–66.5 °C after recrystallization from pentane (lit.[23] mp 74–76 °C), followed by ester **8** and finally 0.092 g (14%) of benzophenone **29.**

Treatment of Enol Lactone 31 with LiH. Enol lactone **31** (0.40 g, 1.31 mmol) and LiH (0.0106 g, 1.33 mmol) were stirred in refluxing THF for 46 h. After evaporation of the solvent, the residue was dissolved in H_2O and extracted with Et_2O. The extract was dried, evaporated, and chromatographed to give 0.345 g (87%) of benzophenone **29.**

Xanthone 30. Treatment of benzophenone **29** (0.119 g, 0.391 mmol) with 10% methanolic KOH (10 ml) for 92 h at 25 °C gave, after dilution with water and acidification, 0.093 g (87%) of xanthone **30.**

Lichexanthone (2). A suspension of xanthone **30** (0.101 g, 0.367 mmol) in 30 ml of Et_2O was treated with excess ethereal CH_2N_2. After TLC indicated disappearance of **30,** the solvent was evaporated in vacuo to give 0.104 g (99%) of **2:** mp 184.5–186 °C after recrystallization from acetone (lit.[3] mp 187 °C); IR (KBr) 1640, 1600, 1565 cm^{-1}; UV 241 nm (ϵ 33 300), 254 (26 200), 308 (21 900), 340 (7800). Anal. Calcd for $C_{16}H_{14}O_5$: C, 67.13; H, 4.93. Found: C, 66.92; H, 4.86.

Treatment of 18 with Methanolic NaOAc followed by HI. Hemiketal **18** (0.0949 g, 0.295 mmol) was treated with 1 M methanolic NaOAc (3 ml) for 23 h at 25 °C to give 0.0546 g (~64%) of the 4′-methyl ether (**32**) of alternariol, mp 259–261.5 °C dec (sealed tube) after recrystallization from EtOH. A portion of the product was collected by filtration and the remainder by extraction after addition of water to the reaction mixture: IR (KBr) 1645, 1601 cm^{-1}; UV 256 nm (ϵ 44 700), 280 (10 200), 300 (11 100), 332 (11 500), 340 (11 200); NMR (C_5D_5N) 2.50 (s, 3, CH_3), 3.73 (s, 3, OCH_3), 6.74 (s, 1, OH), 6.83 (d, 2, aromatic, J = 2 Hz), 7.15 (s, 2, aromatic); MS *m/e* (rel intensity) 272 (100), 243 (2), 229 (4), 187 (11). Crystals retained water and solvents; elemental analyses indicated the presence of at least $1H_2O$. The molecular formula of **32** was established by exact mass measurement. Anal. Calcd for $C_{15}H_{12}O_5$: *m/e* 272.0707. Found: *m/e* 272.0685. Ether **32** (0.011 g, 0.04 mmol) was treated with refluxing constant-boiling HI (2 ml) for 26 h. Addition of the mixture to ice water gave 0.0088 g (90%) of alternariol (**3**), mp 335–337 °C dec after air drying (lit.[5] mp 340 °C).

Treatment of 26 with Methanolic NaOAc. Hemiketal **26** (0.258 g, 0.84 mmol) was treated with 1 M methanolic NaOAc (10 ml) for 8 h at 25 °C. The mixture was added to H_2O and extracted with Et_2O. Extraction of the ethereal solution with aqueous $NaHCO_3$ gave after acidification 0.099 g (62%) of 4,7-dihydroxy-5-methylcoumarin (**34**): mp 268–269 °C after recrystallization from H_2O (lit.[24] mp 266–267 °C); IR (KBr) 1658, 1613, 1550 cm^{-1}; UV 216 nm (ϵ 16 100), 239 (sh, 9900), 312 (14 400); NMR ($CDCl_3$–CD_3SOCD_3) 2.61 (s, 3, CH_3), 3.50 (br, 2, OH), 5.40 (s, 1, vinyl), 6.54 (s, 2, aromatic); MS *m/e* (rel intensity) 192 (parent, 71), 164 (14), 150 (100), 122 (45). The ethereal solution from above contained little or no alternariol (**3**).

Treatment of 26 with Methanolic 4:1 NaOAc–HOAc. Hemiketal **26** (0.204 g, 0.66 mmol) was treated with 10 ml of methanolic NaOAc (1 M) and HOAc (0.25 M) for 5.5 days at 25 °C. After isolation as above, the ethereal solution contained 0.0522 g (31%) of **3:** mp 329–333 °C dec after recrystallization from EtOH–H_2O: IR (KBr) 1655, 1615, 1580 cm^{-1}; UV 257 nm (ϵ 38 900), 290 (9100), 302 (9600), 340 (9800); MS *m/e* (rel intensity) 258 (parent, 100), 230 (8), 229 (7), 130 (5), 69 (10). The aqueous $NaHCO_3$ solution gave after acidification and extraction with Et_2O 0.0641 g (50%) of coumarin **34,** mp 271–273 °C after recrystallization from MeOH–H_2O.

Treatment of 26 with Methanolic 1:1 NaOAc–HOAc. Hemiketal **26** (0.050 g, 0.162 mmol) was treated with 5 ml of methanolic NaOAc (1 M) and HOAc (1 M) for 9 days at 25 °C. Work-up as above gave 0.0219 g (52%) of **3** and 0.0147 g (47%) of **34.**

2-(3-Carbomethoxyacetonyl)-7-hydroxy-5-methylchrom-4-one (36). Hemiketal **26** (0.122 g, 0.396 mmol) was treated with a catalytic amount of *p*-toluenesulfonic acid in refluxing benzene (15 ml) for 12 h to give 0.103 g (90%) of chromone **36:** mp 168–169.5 °C after recrystallization from MeOH–H_2O; IR (KBr) 1740, 1715, 1650, 1625, 1595, 1560 cm^{-1}; UV 244 nm (ϵ 17 700), 252 (18 200), 294 (11 500), 340 (1400); NMR (CD_3SOCD_3) 2.67 (s, 3, CH_3), 3.68 (s, 3, CH_3), 3.77 (s, 2, CH_2), 3.95 (s, 2, CH_2), 6.06 (s, 1, vinyl), 6.61 (s, 2, aro-

matic); MS *m/e* (rel intensity) 290 (parent, 85), 258 (20), 216 (88), 190 (100), 161 (28), 151 (25), 59 (29). Anal. Calcd for $C_{15}H_{14}O_6$: C, 62.07; H, 4.86. Found: C, 61.91; H, 4,86.

Treatment of Chromone 36 with NaOMe. Chromone **36** (0.0656 g, 0.226 mmol) was treated with excess NaOMe in refluxing MeOH for 29 h to give, after dilution with H_2O, extraction into EtOAc, and chromatography, 0.0082 g (19%) of 7-hydroxy-2,5-dimethylchromone: mp 243–248 °C (lit.[22] mp 253–255 °C); IR (KBr) 1660, 1620, 1570–1545 cm^{-1}; UV 242 nm (ϵ 14 900), 251 (16 500), 292 (10 000), 340 (1400); NMR (CD_3COCD_3) 2.29 (s, 3, CH_3), 2.72 (s, 3, CH_3), 5.93 (s, 1, vinyl), 6.67 (s, 2, aromatic).

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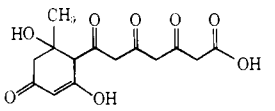

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Clustering of Nitroxide Spin Labels in Lipid Bilayer Membranes

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Abstract: Three new nitroxide (spin-label) biradicals have been prepared: *N,N'*-dipalmitoyl-*N,N'*-bis(1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)-1,10-diaminodecane (**5**); *N,N'*-dimethyl-*N,N'*-dihexadecyl-*N,N'*-bis(1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)-1,10-diammoniumdecane diiodide (**7**); *N,N'*-dipalmitoyl-*N,N'*-bis[*N*-(1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)acetamide-2-yl]-1,10-diaminodecane (**9**). It is shown that these biradicals bind strongly to phosphatidylcholine–cholesterol bilayer membranes and exhibit a degree of clustering in the plane of the membrane that depends on the particular biradical, the concentration of the biradical in the plane of the membrane, the lipid composition of the membrane, and the temperature. These results illustrate one approach to controlling the lateral distribution and motion of membrane components that is relevant to current studies of membrane immunochemistry.

The paramagnetic resonance spectra of amphipathic nitroxide radicals (spin labels)[1] bound to model membranes[2] and to biological membranes can sometimes provide significant biophysical information on local molecular motion, orientation, and lateral diffusion.[3-7] In recent work we have undertaken a study of the immunochemistry of model membranes containing low concentrations of lipid haptens[8-10] and have employed antibodies specifically directed against nitroxide free radical groups such as the 2,2,6,6-tetramethyl-*N*-oxylpiperidine ring.[9-12] The thrust of these studies is to relate the structure of membrane-bound haptens, and their lateral distributions and motions, to various immunochemical reactions. It is possible that such relationships are significant for some of the more subtle membrane recognition problems in cellular immunology. Spin-labeled lipids and other amphipathic molecules are ideally suited for some of these studies, since their resonance spectra provide direct quantitative information on structure, distribution, and motion, and the *same* molecules can interact specifically with components of the immune system. The initial purpose of the present work was to prepare divalent haptens (nitroxide biradicals) to complement our earlier studies using monovalent haptens, particularly spin-