The Influence of Bulky Substituents on the Syntheses of 4-Hydroxy-3,5-dialkyl Flavanoids

J. HOWARD ADAMS

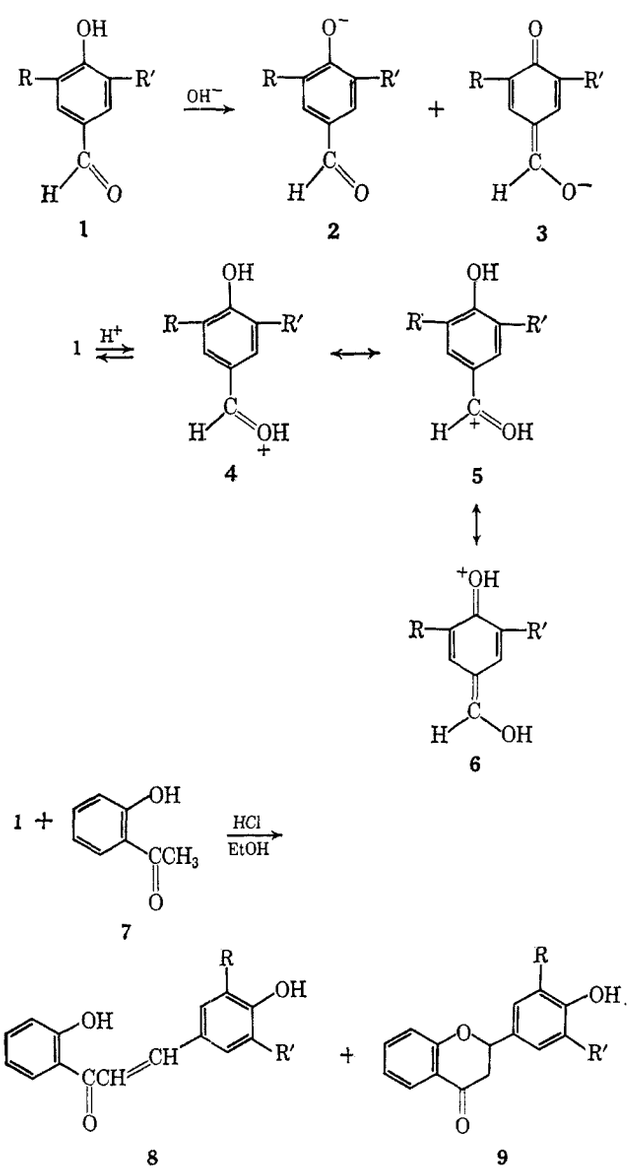
Chevron Research Company, Richmond, California

Received June 16, 1967

A study of the effect of *o*-dialkyl groups on the chemistry of phenolic compounds has led to the synthesis of uniquely substituted flavanoid derivatives. The acid-catalyzed condensation of 4-hydroxy-3,5-dialkylbenzaldehydes with *o*-hydroxyacetophenone has yielded 2′,4-dihydroxy-3,5-dialkylchalcones (**8**) and 4′-hydroxy-3′,5′-dialkylflavanones (**9**), both of which can be oxidized by alkaline hydrogen peroxide to 3,4′-dihydroxy-3′,5′-dialkylflavones (**16**) with the notable exception of the di-*t*-butyl case. The chalcone–flavanone equilibrium in acid is shown to be influenced by the size of the alkyl substituents. All results are interpreted in terms of quinone methide ion intermediates.

Even though innumerable substituted benzaldehydes have been condensed with 2′-hydroxyacetophenones (**7**) to form mixtures of corresponding 2′-hydroxychalcones and flavanones, there is no record of any 4-hydroxy-3,5-dialkylbenzaldehydes (**1**) (Scheme I) having been tried in order to obtain dialkylhydroxychalcones (**8**) and flavanones (**9**). It was of interest to attempt the synthesis of these hindered hydroxy flavanoids and study the effect of the dialkyl groups on the chemistry of this class of compounds that are so common in plants.

SCHEME I



Some departure from normal benzaldehyde behavior was to be expected in the base-catalyzed condensation reaction. Several groups[1–7] have pointed out the substantial influence that bulky *o*-dialkyl groups have in affecting the reactivity of functional groups *para* to the hindered hydroxyl of the phenol in alkaline media. Cohen and Jones[3] explained this phenomena as due to an abnormal increase of negative charge density at the reaction site because of the favoring of an intermediate in a quinone methide anion form (**3**) since it "offers least steric hindrance to ionic solvation."[2]

This paper presents the results observed in the condensation reaction of the 4-hydroxy-3,5-dialkybenzaldehydes with 2′-hydroxyacetophenone and the effect of the 4-hydroxy-3,5-dialkyl substituents on the chemistry of the subsequent chalcones (**8**), flavanones (**9**), and flavonols (**16**).

Results and Discussion

Both acid and base have been employed as catalyst in the condensation reaction of benzaldehyde with acetophenone; the latter is deemed most suitable.[8] Although *p*-hydroxybenzaldehyde (**1a**) condenses readily with *o*-hydroxyacetophenone (**7**) in alkaline media, 4-hydroxy-3,5-dimethylbenzaldehyde (**1b**) reacts in considerably lower conversion, and the other 4-hydroxy-3,5-dialkylbenzaldehydes with bulkier substituents react not at all. Apparently, the predominance of the quinone methide ion (**3**) in the alkaline media prohibited the condensation reaction.[3]

Sipos and Sirokman[9] have shown that *p*-hydroxybenzaldehyde will not condense with hydroxynitroacetophenones in the presence of base but will in acid. The identical situation occurs in this work, as indeed, when the attempted condensations of the benzaldehydes (**1**) (Table I) with **7** were repeated in absolute

(1) L. A. Cohen, *J. Org. Chem.*, **22**, 1333 (1957).
(2) L. A. Cohen and W. M. Jones, *J. Am. Chem. Soc.*, **82**, 1907 (1960).
(3) L. A. Cohen and W. M. Jones, *ibid.*, **84**, 1965 (1962).
(4) L. J. Filar and S. Winstein, *Tetrahedron Letters*, No. 25, 9 (1960).
(5) G. A. Nikiforov and V. V. Ershov, *Izv. Akad. Nauk SSSR Ser. Khim.*, No. **2**, 293 (1964).
(6) T. Coffild, A. Fibley, G. Ecke, and A. Kolka, *J. Am. Chem. Soc.*, **79**, 5019 (1957).
(7) A. Fischer, G. J. Leary, R. D. Topsom, and J. Vaughn, *J. Chem. Soc., Sec. B*, 782 (1966).
(8) T. A. Geissman, "The Chemistry of Flavanoid Compounds," Macmillan and Co., New York, N. Y., 1962, p 308.
(9) Gy. Sipos and F. Sirokman, *Nature*, **202**, 489 (1964).

TABLE I
THE ACID-CATALYZED CONDENSATION OF 4-HYDROXY-3,5-DIALKYLBENZALDEHYDE (1) WITH 2′-HYDROXYACETOPHENONE (7)[a]

Reactant benzaldehyde	R	R′	Reaction temp, °C	Yield,[e] % Chalcone	Flavanone	Arylidene-flavanone
1a	H	H	25[c]	30 (8a)	32 (9a)	36 (11)
1b	Me	Me	0	9 (8b)	19 (9b)	
1c[b]	Me	*t*-Bu	0	58 (8c)		
1d	*i*-Pr	*i*-Pr	0	18 (8d)		
1e	*t*-Bu	*t*-Bu	25[d]	20 (8e)	35 (9e)	20 (10)

[a] For base-catalyzed results see Experimental Section. [b] Recrystallization of the crude product was unsuccessful so it was treated with base to convert the flavanone (9c) present to chalcone (8c). The product then essentially all 8c did crystallize. [c] The reaction carried out at 0° went to completion but no trace of 11 was detected. [d] The reaction carried out at 0° resulted in only about 50% conversion. [e] Infrared analyses of the crude products showed no trace of starting material but low yields were obtained because these flavanoids are difficult to crystallize.

ethanol saturated with hydrogen chloride, success was achieved as mixtures of the corresponding 2′-hydroxychalcones (8) (Table II) and flavanones (9) (Table III)

TABLE II
2′,4-DIHYDROXY-3,5-DIALKYLCHALCONES

No.	R	R′	Mp, °C	Recrystn solvent[d]	Formula	Calcd, % C	Calcd, % H	Found, % C	Found, % H
8b	Me	Me	191–192[a]	A	$C_{17}H_{16}O_3$	76.09	6.01	76.14	6.33
8c	Me	*t*-Bu	156–157[b]	A	$C_{20}H_{22}O_3$	77.38	7.14	77.18	6.91
8d	*i*-Pr	*i*-Pr	145–146[c]	A	$C_{21}H_{24}O_3$	77.74	7.46	77.74	7.31
8e	*t*-Bu	*t*-Bu	173–174[a]	B	$C_{23}H_{26}O_3$	78.37	8.00	78.36	7.91

[a] Yellow needles. [b] Orange plates. [c] Orange-yellow needles. [d] A, methanol–water; B, ether–pentane.

TABLE III
4′HYDROXY-3′,5′-DIALKYLFLAVANONES

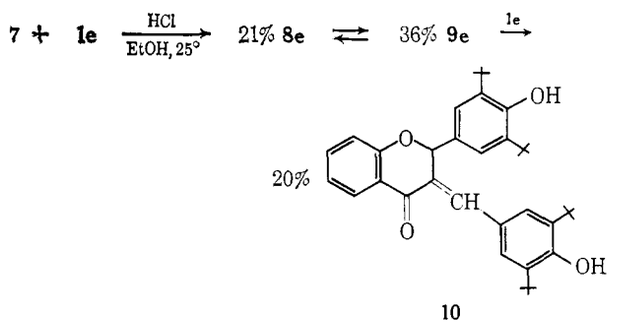
No.	R, R′	Mp, °C	Formula	Calcd, % C	Calcd, % H	Found, % C	Found, % H
9b	Me	174.5–5.5[a]	$C_{17}H_{16}O_3$[c]	76.09	6.01	76.12	6.10
9e	*t*-Bu	139.0–140.5[b]	$C_{23}H_{28}O_3$	78.37	8.00	78.01	7.93

[a] Pale yellow needles from acetone–water. [b] White needles from ether–pentane. [c] Molecular weight 268 determined by mass spectrum.

were obtained in high conversion at temperatures from −10 to 25°. All compounds in the product mixture were separated by fractional crystallization when possible and identified by elemental analysis, infrared, and nuclear magnetic resonance (nmr) spectra.

A probable explanation[9] as to why the *p*-hydroxybenzaldehydes (1) condense so readily with 7 in acid media is that the reactive benzenoid tautomers 4 and 5 are favored over the unreactive quinoid form (6). The nonaromatic form (6) is not only less stable electronically, but its formation would also be sterically hindered when the alkyl groups are large.

Arylideneflavanones.—All of the *p*-hydroxybenzaldehydes (1) condense with 7 readily under acid catalysis at ice-bath temperature with the exception of 1e, which requires a higher temperature of 25°. At this temperature, a competitive condensation reaction takes place between the flavanone (9e) and the benzaldehyde (1e), resulting in a 20% yield of 3-(4-hydroxy-3,5-di-*t*-butylbenzylidene)-4′-hydroxy-3′,5′-di-*t*-butylflavanone (10) when equimolar amounts of 1e and 7 are combined. 3-Benzylideneflavanones have



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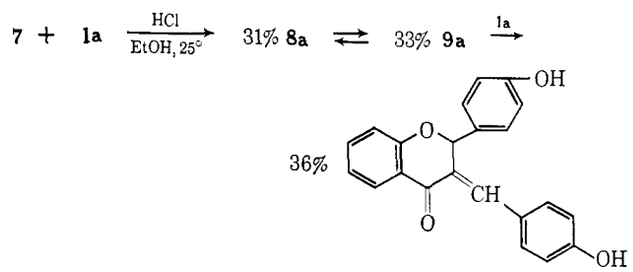
been isolated in condensation reactions of benzaldehyde with substituted *o*-hydroxyacetophenones only when a procedure involving long reaction times or high temperatures plus an excess of benzaldehyde was employed.[10,11]

The increase in ease of condensation of 1e with the flavanone (9e) in this work is deemed due not to the alkyl groups but to the *p*-hydroxy group of the benzaldehyde causing the aldehyde to be more electrophilic. This explanation is based on the observation that when the reaction of 1a with 7 is carried out at 25° instead of at 0°, 3-(4-hydroxybenzylidene)-4′-hydroxyflavanone (11) (Table IV) is isolated in the product mixture and in greater yield than the amount of 10 that was produced from the combination of 1e and 7.



Chalcone–Flavanone Equilibria in Acid.—The relative amounts of the chalcones (8) and flavanones (9) prepared from the acid-catalyzed condensation method appeared to vary depending on the size of their alkyl groups. Consequently, a study was undertaken to determine the exact magnitude of this influence.

Table V lists the relative percentages of chalcone and flavanone determined after the mixture had reached equilibrium in an absolute ethanol–hydrogen chloride solution. An infrared spectrum was then taken of the mixture and the carbonyl absorbance bands of the chalcone and flavanone (~50 cm^{-1} apart) were measured. The relative amounts of the two compounds were calculated (±2%) from the determined relative molar absorbances (±0.5%) (see Experimental Section). Although the effects of the different alkyl groups shown in Table V do not show any of the sharp limiting differences noted for *o*-dialkyl phenolics in alkaline solutions,[5,6] a definite influence, though minor, of the alkyl group size on the hydroxychalcone–flavanone equilibrium in acid can be observed. It is proposed that the equilibrium is controlled by the relative importance of the tautomers 14 and 15.

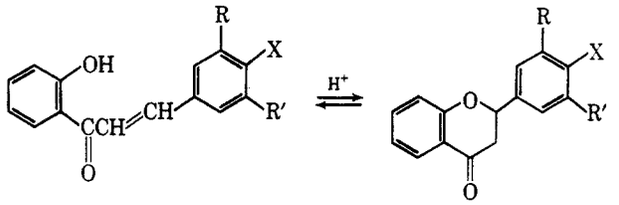
(10) M. K. Seikel, M. J. Lounsbury, and S. Wang, *J. Org. Chem.*, **27**, 2952 (1962).
(11) T. Széll and R. É. M. Unyi, *ibid.*, **28**, 1146 (1963).

TABLE IV
3-(4-HYDROXY-3,5-DIALKYLBENZYLIDENE)-4′-HYDROXY-3′,5′-DIALKYLFLAVANONES

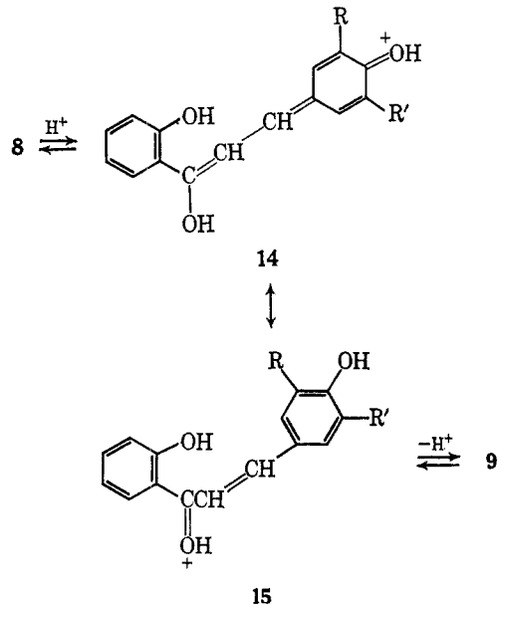
No.	R, R′	Mp, °C	Formula	Calcd, % C	Calcd, % H	Found, % C	Found, % H	Mol wt, mass spectrum	Infrared, cm⁻¹ >C=O	Infrared, cm⁻¹ OH	Phase
11	H	216–217[a]	$C_{22}H_{16}O_4$	76.73	4.68	76.63	4.61	344	1650	3320	KBr
10	*t*-Bu	206–207[b]	$C_{38}H_{48}O_4$	80.24	8.51	80.47	8.31	568	1670	3620	CCl_4

[a] Clear light yellow prisms from benzene–hexane. [b] Beige rhomboid prisms from ether–pentane.

TABLE V
INFRARED DETERMINATION OF CHALCONE–FLAVANONE EQUILIBRIUM



X	R	R′	Infrared medium	Chalcone No.	Chalcone Carbonyl frequency, cm⁻¹	Chalcone Carbonyl absorb	Chalcone % at equil	Flavanone No.	Flavanone Carbonyl frequency, cm⁻¹	Flavanone Carbonyl absorb	Flavanone % at equil	Flavanone–chalcone molar absorb ratio
H	H	H	CCl_4	**12**	1645	0.088	17	**13**	1695	0.38	83	0.90
OH	H	H	KBr	**8a**	1630	0.28	47	**9a**	1670	0.49	53	1.53
OH	CH_3	CH_3	KBr	**8b**	1635	0.51	59	**9b**	1685	0.46	41	1.29
OH	CH_3	*t*-Bu	CCl_4	**8c**	1635	0.28	44	**9c**	1695	0.19	56	0.53
OH	*i*-Pr	*i*-Pr	CCl_4	**8d**	1640	0.29	42	**9d**	1695	0.175	58	0.44
OH	*t*-Bu	*t*-Bu	CCl_4	**8e**	1640	0.34	39	**9e**	1700	0.34	61	0.62



Under the acid conditions employed, the equilibrium of unsubstituted 2′-hydroxychalcone (**12**) and flavanone (**13**) is in favor of the cyclized form **13** by a 17 to 83 ratio. By the addition of a *p*-hydroxyl group in the "B" ring the ratio is dropped closer to equality, 47% **8a** to 53% **9a**. The hydroxyl group has acted to stabilize the chalcone ion structure by making possible the presence of two resonating chalcone forms, the quinoid ion (**14**) and the benzenoid ion (**15**).

When two methyl groups are introduced, *ortho* to the hydroxyl group in the "B" ring, the chalcone (**8b**)–flavanone (**9b**) is shifted in favor of the chalcone by a 59 to 41 ratio. The methyl groups act to feed still more electrons to the resonating **14** and **15**, thus further stabilizing the chalcone form.

For larger alkyl groups than methyl, the quinone methide tautomer **14** falls into disfavor because of a steric hindrance to the solvated ion. A shift to the sterically unhindered tautomer ion **15** then leads to an increase in flavanone in the equilibrium. Thus, when one alkyl group is methyl and the other *t*-butyl, the flavanone (**9c**) concentration increases to 56%. When the alkyls are both isopropyl, the flavanone (**9d**) commands 58% of the equilibrium. And when the alkyl groups are *t*-butyl, the equilibrium ratio of flavanone (**9e**) to chalcone (**8e**) reaches 61–39%.

This analytical technique should be of general utility for studying the effect of other substituents either encouraging or discouraging ring closure of the chalcone to the γ-pyrone ring form of the flavanone. Varying substituents with large differences in Hammett σ values should show more clearly the influence of electronic effects on chalcone–flavanone equilibria.

AFO Oxidation of 2,4′-Dihydroxy-3,5-dialkylchalcones (8).—The base-catalyzed hydrogen peroxide oxidation of 2′-hydroxychalcones to flavonols (3-hydroxyflavones), commonly called the Algar–Flynn–Oyamada (AFO) reaction,[12] also yields several other types of compounds depending on the position of substituents on the aromatic rings.[13]

The AFO oxidation of **8a** results in a mixture of 3,4′-dihydroxyflavone (**16a**) and 2-hydroxyphenylbenzofuran-3-carboxylic acid (**17**) as previously reported.[13] The same oxidation of **8b–d** results in the formation of only the 3,4′-dihydroxy-3′,5′-dialkylflavone (**16b–d**) in good yield and identified by elemental analysis, infrared, and nmr spectra (Tables VI and VII). Apparently the presence of the alkyl groups increases the specificity of the reaction.

(12) J. Algar and J. P. Flynn, *Proc. Roy. Irish Acad.*, **42B**, 1 (1934); T. Oyamada, *J. Chem. Soc. Japan*, **55**, 1256 (1934); *Bull. Chem. Soc. Japan*, **10**, 182 (1935).

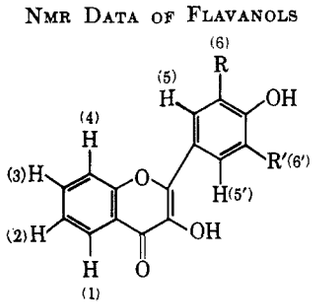
(13) B. Cummins, D. M. X. Donnelly, J. F. Eades, H. Fletcher, F. O'Cinneide, E. M. Philbin, J. Swirski, T. S. Wheeler, and R. K. Wilson, *Tetrahedron*, **19**, 499 (1963).

TABLE VI
3,4'-DIHYDROXY-3',5'-DIALKYLFLAVONES

No.	R	R'	Mp, °C[a]	Yield,[d] %	Infrared, cm⁻¹ >C=O	Infrared, cm⁻¹ OH	Phase	Formula	Calcd, % C	Calcd, % H	Found, % C	Found, % H
16b	Me	Me	224–225[b]	84	1610	3290	KBr	$C_{17}H_{14}O_4$	72.32	5.00	72.11	5.12
16c	Me	*t*-Bu	204–205[c]	62	1615	3590, 3370	$CHCl_3$	$C_{20}H_{20}O_4$	74.04	6.21	73.78	6.13
16d	*i*-Pr	*i*-Pr	174–175[b]	88	1615	3605, 3370	CCl_4	$C_{21}H_{22}O_4$	74.53	6.55	74.50	6.85
16e	*t*-Bu	*t*-Bu										

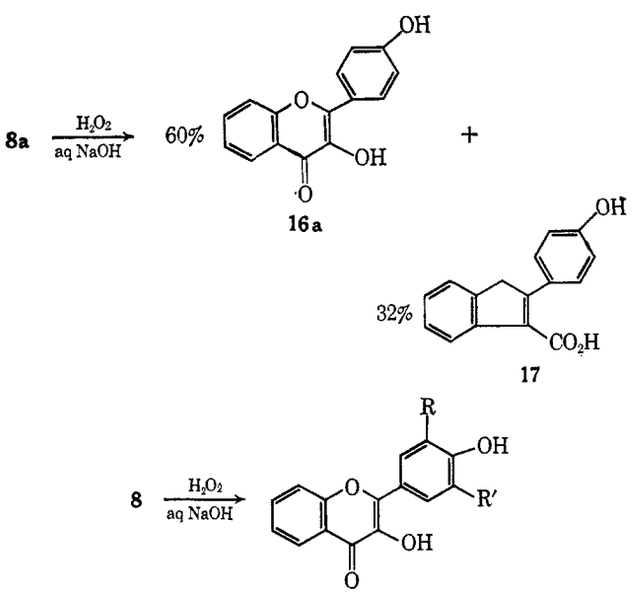
[a] Recrystallized from methanol. [b] Pale yellow needles. [c] Yellow globules. [d] Infrared analysis of crude product could not detect any other product or starting material.

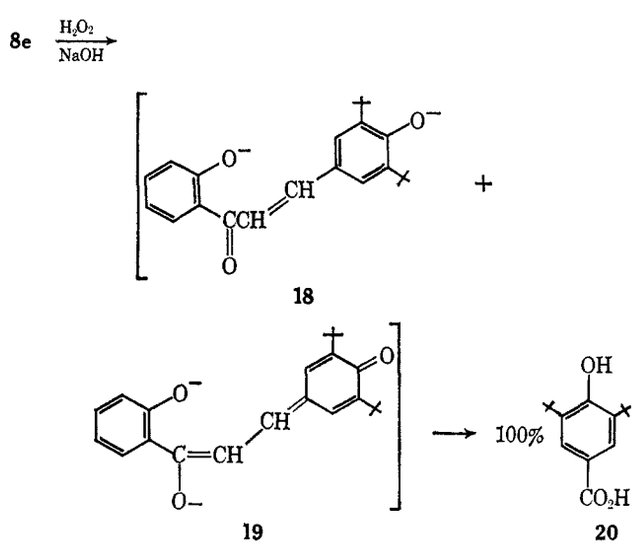
TABLE VII
NMR DATA OF FLAVANOLS



	Ppm values (*J*, cps) R = H (16a)[a]	R = CH_3 (16b)[a]	R = CH_3, *t*-Bu (16c)[b]	R = *i*-Pr (16d)[b]
Proton				
1 q (~8.0; ~2.0)	8.10	8.16	8.18	8.20
2,3,4 unresolvable multiplets	7.2–7.7	7.2–7.7	7.2–7.7	7.2–7.7
5	8.06 d (~9.0)	7.82 s	(5) 7.88 d (~3.0); (5') 8.06 d (~3.0)	7.95 s
6	6.88 d (~9.0)	2.29 s	(6) 2.32 s; (6') 1.42 s	3.23 p (~7.0); 1.32 d (~7.0)
OH			6.25 s (br)	5.70 s (br)

[a] At 60°, $CDCl_3$ + DMSO-d_6. [b] At 40°, $CDCl_3$.

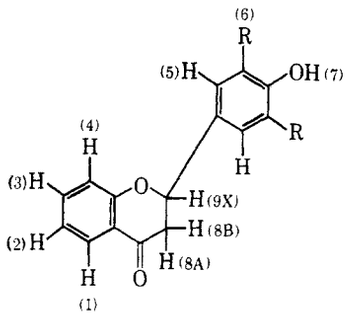




A remarkably different result occurs when the AFO reaction is carried out with **8e**. 4-Hydroxy-3,5-di-*t*-butylbenzoic acid (**20**) is the only water-insoluble product obtained and in quantitative yield identified by nmr, mass spectroscopy, and infrared analysis. The only difference noted in the reaction of **8e** compared to those of **8b–d** was that **8e** is partially soluble, whereas the others are totally soluble in aqueous sodium hydroxide. The solubility decrease is undoubtedly due to the steric hindrance to the formation of the solvated anion **18**. The addition of a small amount of methanol readily brings **8e** into solution for the AFO reaction to be carried out. To check whether a solvent effect is responsible, the AFO reaction was run on **8d** also adding some methanol to the solution in identical fashion as with **8e**. Again, **16d** was the principal product observed. Using a smaller amount of hydrogen peroxide in the reaction with **8e** yielded only **20** and starting material.

Although benzoic acids are commonly isolated in AFO reaction work-ups and the occurrence of one would not be unexpected, it is extraordinary that such a critical limiting case occurs between the otherwise chemically similar **8d** and **e**. As previous workers have shown for other examples of chemical differences

TABLE VIII
NMR DATA OF FLAVANONES



	Ppm values (J, cps)		
	R = H (9a)[a]	R = CH_3 (9b)[b]	R = t-Bu (9e)[c]
Proton number			
1 q (~8.0; ~2.0)	7.81	7.84	7.76
3 three doublets ($J_{3,4} = J_{3,2}$ ~8.0; $J_{1,3} \simeq 2.0$)	7.37	7.37	7.30
2,4 unresolvable multiplets at 6.8–7.0			
5	6.79 d (~8.0)	6.98 s	7.13 s
6	7.20 d (~8.0)	2.20 s	1.43 s
7 s	8.37	4.68	5.23
8A q	3.02 (J_{AX} ~12.0; J_{AB} ~17.0)	3.02 (J_{AX} ~12.0; J_{AB} ~17.0)	2.97 (J_{AX} = 12.4; J_{AB} = 15.9)
8B q	2.73 (J_{BX} ~4.0; J_{AB} ~17.0)	2.73 (J_{BX} ~4.0; J_{AB} ~17.0)	2.66 (J_{BX} = 3.3; J_{AB} = 15.9)
9X q	5.29 (J_{AX} ~12.0; J_{BX} ~4.0)	5.29 (J_{AX} ~12.0; J_{BX} ~4.0)	5.23 (J_{AX} = 12.4; J_{BX} = 3.3)

[a] At 60°, $CDCl_3$ + 1 drop of DMSO-d_6. [b] At 60°, $CDCl_3$. [c] At 30°, CCl_4.

between *o*-di-*t*-butyl- and *o*-diisopropyl-substituted phenols[6] and *p*-hydroxybenzaldehydes,[5] the reason must involve the larger *t*-butyl groups strongly favoring the quinone anion intermediate **19**. Otherwise, the phenolate ion **18** would become oxidized by the hydrogen peroxide to the flavonol (**16e**) according to the currently accepted mechanism for the AFO oxidation.[13,14]

Experimental Section

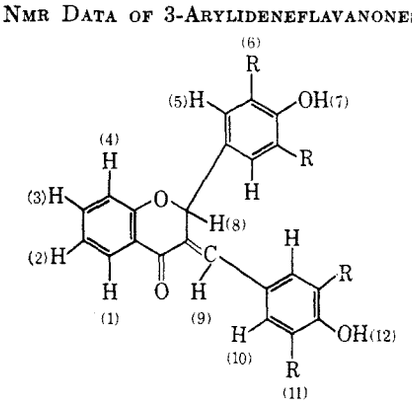
The melting points are corrected and were obtained on a Laboratory Devices Mel-Temp integrated capillary apparatus. The infrared spectra were obtained using a Perkin-Elmer 337 grating infrared spectrometer. The carbonyl frequencies of the chalcones and flavanones are presented in Table V. The nmr spectra were measured on a Varian Associates A-100 instrument and tetramethylsilane as an internal standard. The nmr data of the flavanoids are presented in Tables VII–X. The mass spectra were determined with an AEI MS-9 instrument. The microanalyses were performed by L. L. Farley and his associates at this laboratory.

The Preparations of the 4-hydroxy-3,5-dialkylbenzaldehydes (**1b–e**) were carried out from the corresponding 2,6-dialkylphenols according to the method of Nikoforov and co-workers giving satisfactory yields and melting points in accord with those reported.[15]

The preparation of 2′-hydroxychalcone (**12**) was carried out according to the method of Feuerstein and Kostanecki[16] in 50% yield and mp 87–88° (lit.[16] 88–89°). This chalcone in turn was converted to **flavanone** (**13**) according to the method of Kostanecki and Szabranski:[17] white needles, mp 75–76° (lit.[17] mp 75–76°).

The Base-Catalyzed Condensation of *p*-Hydroxybenzaldehyde (**1a**) **with *o*-Hydroxyacetophenone** (**7**).—A mixture of 8.0 g **1a** and 3.6 g of **7** was treated according to the method of Simpson and Whalley[18] to yield 3.45 g (62% of theory) of **2′,4-dihydroxychalcone** (**8a**), mp 157.5–159.0° (lit.[19] mp 162.0–162.5°), and 0.50 g (9% of theory) of **4′-hydroxyflavanone** (**9a**), mp 186–188° (lit.[19] mp 186–187°).

TABLE IX
NMR DATA OF 3-ARYLIDENEFLAVANONES



	Ppm values (J, cps)	
	R = H (11)[a]	R = t-Bu (10)[b]
Proton number		
1	7.81 d (~8.0)	7.88 d (~8.0)
2	6.84 t (~8.0)	6.85 t (~8.0)
3	7.25 t (~8.0)	7.29 t (~8.0)
4	6.80 d (~8.0)	6.81 d (~8.0)
5	7.08 d (~8.0)	7.03 s
6	6.92 d (~8.0)	1.26 s
7	8.92 s (br)	5.11 s
8	6.51 s	6.48 s
9	7.90 s	8.03 s
10	7.20 d (~8.0)	7.20 s
11	6.74 d (~8.0)	1.31 s
12	9.45 s (br)	5.42 s

[a] At 30°, $CDCl_3$ + 1 drop of DMSO-d_6. [b] At 30°, $CDCl_3$.

The Base-Catalyzed Condensation of 4-Hydroxy-3,5-dimethylbenzaldehyde (**1b**) **with *o*-Hydroxyacetophenone** (**7**).—A mixture of 480 mg (3.2 mmoles) of **1b** and 440 mg (3.2 mmoles) of **7** was dissolved in 6 ml of ethanol at ice-bath temperature and 5.5 ml of 60% potassium hydroxide (aqueous) was added

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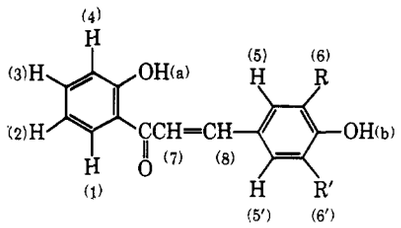
TABLE X
NMR DATA OF CHALCONES



	Ppm values (J, cps)				
	R = H (8a)[b]	R = CH_3 (8b)[a]	R = CH_3 and t-Bu (8c)[a]	R = i-Pr (8d)[c]	R = t-Bu (8e)[a]
Proton number					
1 d (~8.0)	7.86	7.87	7.87	7.86	7.88
2 t (~8.0)	6.86	6.84	6.84	6.82	6.86
3 t (~8.0)	7.40	7.38	7.39	7.38	7.38
4 d (~8.0)	6.92	6.88	6.92	6.92	6.89
5	7.48 d (~8.0)	7.21 s	7.28 s (5); 7.37 s (5')	7.18 s	7.44s
6	6.84 d (~8.0)	2.21 s	2.25 s (6); 1.41 s (6')	3.17 p (~7.0); 1.33 d (~7.0)	1.44s
7 d (~15.0)	7.42	7.40	7.41	7.41	7.42
8 d (~15.0)	7.82	7.75	7.83	7.84	7.86
a s	12.96	12.96	12.93	12.83	12.98
b s	9.3	8.0	6.44	5.15	6.04

[a] At 35°, $CDCl_3$ + 1 drop of dimethyl sulfoxide-d_6 (DMSO-d_6). [b] At 30°, same as a. [c] At 35°, CCl_4.

slowly. The reaction mixture was stirred for 2 days at room temperature, then poured into cold dilute hydrochloric acid. The resulting precipitate was suction filtered, water washed, and recrystallized from methanol–water to yield 300 mg (35% of theory) of yellow needles identified by infrared and nmr spectra as 2',4-dihydroxy-3,5-dimethylchalcone (8b).

The second crop was 300 mg (63% of theory) of impure starting material 1b, mp 97–103°. The infrared spectrum was identical with that of an authentic sample.

The base-catalyzed condensation of 4-hydroxy-3,5-diisopropylbenzaldehyde (1d), 4-hydroxy-3-methyl-5-*t*-butylbenzaldehyde (1c), and 4-hydroxy-3,5-di-*t*-butylbenzaldehyde (1e) with *o*-hydroxyacetophenone trying all of the previously described procedures[18,19] resulted in recovery of only starting material as identified by infrared analysis.

The General Procedure for the Acid-Catalyzed Condensation of 4-Hydroxy-3,5-dialkylbenzaldehydes (1) with *o*-Hydroxyacetophenone (7).—Hydrogen chloride was bubbled through 75 ml of absolute ethanol for 10 min. When the solution cooled to room temperature, 5.0 mmoles of 1 and 5.0 mmoles of 7 were added and the reaction mixture was stirred for 2 hr before being poured into ice water. The aqueous solution was extracted three times with ether. The ether extracts were washed twice with water and evaporated down to a 10-ml solution. Pentane was added for recrystallization. The chalcones (8), flavanones (9), and arylideneflavanones (10 and 11) (Tables II–IV) were isolated in varying yields depending on temperature and the alkyl substituents as shown in Table I. The infrared and nmr spectra of the compounds were consistent with the assigned structure.

The Determination of the Equilibrium Ratio of 2'-Hydroxychalcone (12) and Flavanone (13) in Acid.—Hydrogen chloride was bubbled through 25 ml of absolute ethanol at a rapid rate for 5 min. The solution was warmed briefly to dissolve 100 mg of 12 that was added, let stand at room temperature for 2 hr, and then poured into 250 ml of ice water. The resulting precipitate was suction filtered, washed, dried, and dissolved in carbon tetrachloride. The infrared spectrum showed the presence of both 12 and 13. The carbonyl absorbance of each was determined and recorded in Table I. The chalcone absorbance at 1645 cm^{-1} was 0.088 and the flavanone at 1695 cm^{-1} was 0.38.

In order to determine the relative amounts of 12 and 13, 8.6 mg of 12 and 17.0 mg of 13 were dissolved in carbon tetrachloride. The infrared analysis of the solution showed an absorbance of 0.152 for 12 and 0.280 for 13. The relative molar intensity of flavanone (13) to chalcone (12) was then calculated to be 0.90. The carbonyl absorbance ratio of the equilibrium mixture was adjusted accordingly: (0.088 × 0.90): 0.38 or 0.079:0.38, which is equivalent to the molar ratio 17:83 in favor of flavanone (13).

The determination of the equilibrium ratios of 2',4-dihydroxychalcone (8a)–4'-hydroxyflavanone (9a), 2',4-dihydroxy-3,5-dimethylchalcone (8b)–4'-hydroxy-3',5'-dimethylflavanone (9b), and 2',4-dihydroxy-3,5-di-*t*-butylchalcone (8e)–4'-hydroxy-3',5'-di-*t*-butylflavanone (9e) in acid was carried out according to the preceding procedure. The data obtained are presented in Table XI and in Table V.

TABLE XI
CALIBRATION DATA OF CHALCONE–FLAVANONE MOLAR ABSORBANCES

	Chalcone		Flavanone	
Chalcone–flavanone	Wt, mg	Absorb	Wt, mg	Absorb
8a–9a	5.3	0.105	11.8	0.36
8b–9b	5.7	0.46	6.9	0.72
8e–9e	4.2	0.57	5.3	0.45

The determination of the equilibrium ratio of 2',4-dihydroxy-3-methyl-5-*t*-butylchalcone (8c) and 4'-hydroxy-3'-methyl-5'-*t*-butylflavanone (9c) was carried out according to the previously described procedure with one change. Since no pure 9c was isolated, the calibration had to be determined by a mixture method. As seen in Table V, the infrared carbonyl absorbance for 8c (1635 cm^{-1}) was 0.28 and for 9c (1695 cm^{-1}) was 0.19 in the equilibrium mixture. Exactly 29.6 mg of pure 8c plus 45.4 mg of the above equilibrium mixture dissolved in carbon tetrachloride gave an infrared spectrum with the 8c carbonyl absorbance measuring 0.62 and the 9c carbonyl absorbance measuring 0.17. Therefore, 0.19:0.28 = 0.17:x, when x = 0.25 absorbance units caused by chalcone 8c in the 45.4-mg sample of the mixture. Thus, 29.6 mg of 8c caused 0.62 − 0.25 = 0.37 absorbance units (29.6 mg:0.37 = x':0.25, x' = 20.0 mg of chalcone (8c) in the 45.4-mg mixture sample which then calculates to 44% 8c to 56% 9c).

The relative intensity (Y) of 9c carbonyl absorbance to 8c is, therefore, (44/0.28) = (56/0.19)(Y), Y = 0.53.

The determination of the equilibrium ratio of 2',4-dihydroxy-3,5-diisopropylchalcone (8d)–4'-hydroxy-3',5'-diisopropylflavanone (9d) was carried out in identical fashion with the preceding experiment since 9d was not obtained in pure form. A carbon tetrachloride solution of 9.2 mg of pure 8d and 13.2 mg of the equilibrium mixture of 8d and 9d gave an infrared spectrum with a chalcone carbonyl absorbance of 0.61 and a flavanone carbonyl absorbance of 0.14. From the equilibrium absorbances recorded in Table V, it was calculated (in identical fashion with the preceding experiment) that the equilibrium mixture contained 42% chalcone (8d) and 58% flavanone (9d).

The Algar–Flynn–Oyamada (AFO) oxidation of 2',4-dihydroxychalcone (8a) was carried out on 2.0 g (8.3 mmoles) of 8a

according to the procedure of Cummins, *et al.*,[13] to yield 1.2 g (58% of theory) of 3,4'-dihydroxyflavone (**16a**), mp 275–276° (lit.[20] mp 276°), and 670 mg (32% of theory) of **2-hydroxyphenylbenzofuran-3-carboxylic acid** (**17**), decomposing at 211°, $\nu_{C=O}$ 1685 cm^{-1} (lit.[13] mp 221°, $\nu_{C=O}$ 1686 cm^{-1}).

3,4'-Dihydroxy-3',5'-dialkylflavones (16b–d). General Procedure.—A solution of 0.90 mmole of 2',4-dihydroxy-3,5-dialkylchalcone (**8**) and 5 ml of 10% aqueous sodium hydroxide was cooled to ice-bath temperature in a 25-ml erlenmeyer flask. A 3% aqueous solution of hydrogen peroxide (3.4 ml, 3 mmoles) was added and the reaction mixture was allowed to warm to room temperature, let stand for 3 hr, and then poured into cold dilute hydrochloric acid. The resulting percipitate was suction filtered, water washed, dried, and recrystallized from methanol to give in good yields only the 3,4'-dihydroxy-3',5'-dialkylflavones (**16b–d**) (Table VI).

The AFO Oxidation of 2',4-Dihydroxy-3,5-di-*t*-butylchalcone (8e).—A solution of 500 mg (1.4 mmoles) of 2',4-dihydroxy-3,5-di-*t*-butylchalcone (**8e**), 10 ml of 10% aqueous sodium hydroxide, and 3 ml of methanol was heated until **8e** dissolved, then cooled to 0°. A solution of 3% aqueous hydrogen peroxide (6 ml, 5 mmoles) was added and the mixture was allowed to stand overnight at room temperature. The work-up according to the previously described procedure without recrystallization yielded 350 mg (quantitative) of crude **4-hydroxy-3,5-di-*t*-butylbenzoic acid** (**20**), pearl-colored flakes, mp 205–215° (lit.[21] mp 217–218°).

The infrared spectrum (CCl_4) contained a sharp band at 3620 (hindered phenolic); a strong sharp band at 1680 (aromatic acid C=O); and a sharp band at 1600 cm^{-1} (aromatic C=C). The nmr spectrum ($CDCl_3$) contained sharp singlets at 1.40 (18 H, alkyl CH), 5.63 (1 H, phenolic O—H), and 7.78 (2 H, aryl CH) ppm. The carboxylic acid proton could not be detected.

The mass spectrum of the crude product at high resolution and low voltage showed a molecular ion peak at *m/e* 250 corresponding to **20** and about 1% each of a peak at *m/e* 352 (**8e** or **9e**) and *m/e* 570.336 (unidentified).

Registry No.—**8a**, 13323-66-5; **8b**, 2631-01-8; **8c**, 14919-43-8; **8d**, 14929-97-6; **8e**, 14919-44-9; **9a**, 6515-37-3; **9b**, 2525-91-9; **9e**, 14919-46-1; **10**, 14919-47-2; **11**, 14919-48-3; **16a**, 14919-49-4; **16b**, 14919-50-7; **16c**, 14919-51-8; **16d**, 14919-52-9; **20**, 1421-49-4.

Acknowledgments.—The author wishes to thank Dr. R. M. Teeter for determining the mass spectra, Dr. L. P. Lindeman for determining the nmr spectra, and Dr. S. Winstein for helpful discussion.

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The Structure of Plasmalogens. VIII. Preparation and Properties of Lysophosphatidal Choline[1a]

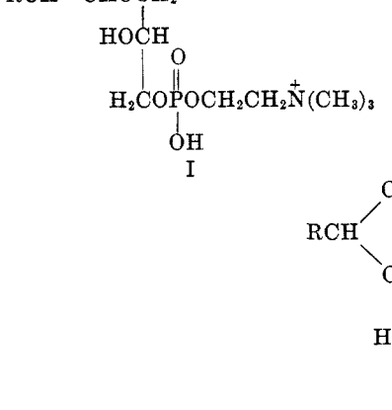
M. F. FROSOLONO,[1b] ALEMKA KISIC, AND MAURICE M. RAPPORT[1c]

Department of Biochemistry, Albert Einstein College of Medicine, Yeshiva University, Bronx, New York

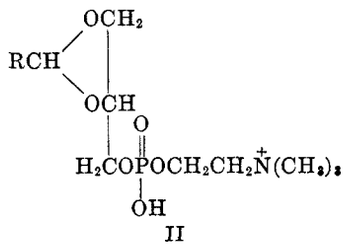
Received June 20, 1967

Preparations of crystalline lysophosphatidal choline were obtained in good yield from beef heart lecithin and physically characterized by means of the molar absorbancy index in chloroform at 6.02 μ and the optical rotation at various wavelengths in both methanol and chloroform solutions. The infrared absorption spectrum provides conclusive evidence that the choline plasmalogen isolated from sea anemones by Bergmann and Landowne was an α,β-unsaturated ether and not a cyclic acetal.

Although lysophosphatidal choline (I) has been used in a number of studies, namely, elucidation of the



I



II

α,β-unsaturated ether structure of plasmalogens,[2] inhibition of respiration of spermatozoa,[3] detection of microsomal lysoplasmalogenase,[4] lysis of erythrocytes,[3,5] and studies of the acyl transferase of liver[6] and erythrocytes,[7] details of the method of preparation of the crystalline compound have appeared only once.[3] In that report, the yield was poor. Crystalline lysophosphatidal choline (190 mg) was obtained from 5.2 g of beef heart lecithin. No physical characterization of the pure substance was presented. Lysophosphatidal choline is an important intermediate for several kinds of studies: comparison of its chemical properties with those of other α,β-unsaturated ethers and comparison of its biochemical properties with those of lysophosphatidyl choline (the acyl ester analog) and lysophosphatidalkyl choline (the saturated ether analog). It is the purpose of this paper to report a convenient method of preparation of lysophosphatidal choline in good yield and to provide a physical characterization of the product from its infrared absorption spectrum and its rotation in two solvents at a series of wavelengths. The characterization by infrared absorption has provided conclusive evidence that the choline plasmalogen isolated from sea anemones by Bergmann and Landowne (and reported in this journal[8]) was incorrectly designated by them to have the structure of a cyclic acetal of glycerol[2] and

(1) (a) This work was supported by grants from the U. S. Public Health Service, (NB-01570 and 5 T1MH-6418); (b) Research Fellow, Interdepartmental Institute for Training in Research in the Behavioral and Neurologic Sciences, Albert Einstein College of Medicine; (c) American Cancer Society Professor of Biochemistry.

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