Benzodipyran Derivatives with Antiallergic Activity

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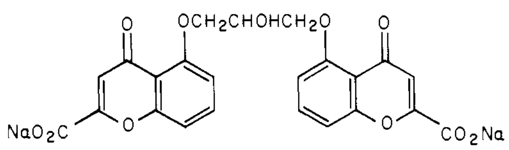
The synthesis and antiallergic activity of a number of benzodipyrandicarboxylic acids are described. Antiallergic activity of the compounds was determined using the homologous passive cutaneous anaphylaxis (PCA) reaction in the rat. The structural requirements for activity in the PCA screen are discussed. In this test system the linear benzodipyrans are more active than their angular analogues.

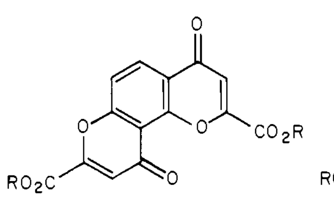
Since the publication of the paper on the chemistry and structure–activity studies on a series of compounds related to disodium cromoglycate (I),[1] several papers, describing other compounds which are potentially useful antiallergic drugs, have appeared. The types of structure for which this activity has been claimed include other chromones,[2-4] xanthones,[5,6] quinoline derivatives,[7] a phenanthroline,[8] 8-azapurin-6-ones,[9] 4-oxo-4*H*-[1]benzothieno[3,2-*b*]pyran-2-carboxylic acid and 4-oxo-4*H*-[1]benzofuro[3,2-*b*]pyran-2-carboxylic acid,[10] 2-nitroindandiones,[11] and 4-hydroxy-3-nitrocoumarins.[12]



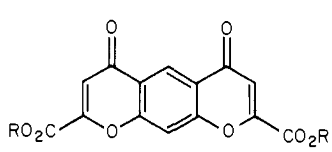
I

This present paper identifies another series of oxygen heterocyclic compounds, the dicarboxybenzodipyrans, which possesses antiallergic activity as demonstrated by their activity in the rat passive cutaneous anaphylaxis test (PCA).[13] Compound **1** has also been shown to inhibit anaphylactic bronchoconstriction in a number of asthmatic human volunteers in experimental antigen provocation tests.[14]

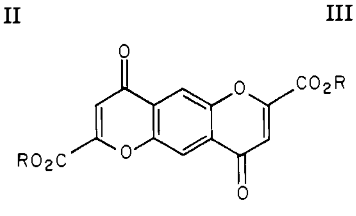
The synthesis and biological activity of substituted derivatives of three skeletal variants (types II–IV, R = H) of the benzodipyran molecule are discussed in this paper.



II



III

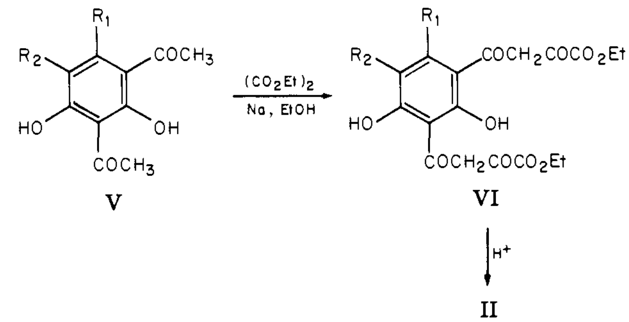


IV

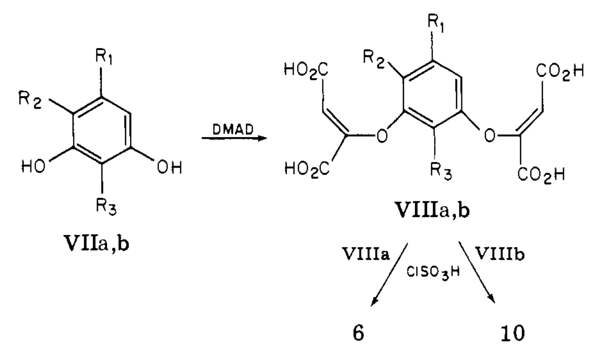
The study of these compounds was undertaken because they embodied several of the features which we believe contribute to the interesting biological activity so clearly demonstrated from the study of disodium cromoglycate and its analogues.[1] Among such features can be listed (i) the presence of two strongly acidic groups (see Experimental Section), (ii) the benzopyran ring system with its 4-oxo group, and (iii) the planarity of the molecules.

Chemistry. The dicarboxybenzodipyrans were usually prepared by the same general procedure. Thus, compounds of formula II were prepared via a double Claisen condensation between the diacetyldihydroxybenzenes V and diethyl oxalate, followed by acid catalyzed cyclization of the α,γ-diketo esters VI as shown in Scheme I (see method A). The compounds of formula III and IV were obtained in similar manner from the appropriately substituted diacetyldihydroxybenzenes. Choice of the final workup conditions gave either the required diacid (R = H) or the corresponding diethyl ester (R = Et). The diesters were readily hydrolyzed to the diacids with $NaHCO_3$ in aqueous EtOH.

Scheme I



Scheme II

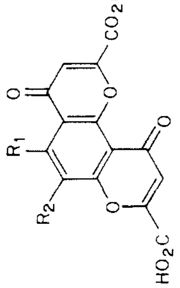


a, $R_1 = R_3 = H$; $R_2 = Cl$
b, $R_1 = Me$; $R_2 = H$; $R_3 = Et$

The only exceptions to this general method were the syntheses of compounds **6**, **10**, and **2**. Compounds **6** and **10** were prepared from the appropriately substituted resorcinol VII via double condensation with dimethyl acetylenedicarboxylate (DMAD), followed by cyclization of the derived fumaric acid VIII with chlorosulfonic acid, as shown in Scheme II (see method B). Compound **2** was prepared by demethylation of compound **1** with HBr–AcOH (see method C).

Of the compounds shown in Tables I–III, **2–4**, **6–8**, **10**, **11**, **21**, and **22** were obtained directly as the free diacids, while compounds **5**, **9**, and **12–20** were isolated initially as the corresponding diethyl esters. Compound **1** was obtained directly as the free acid and also by isolation of its diethyl ester. All the diesters were hydrolyzed to the diacids, except those of compounds **5** and **16–18**, which were converted directly into their respective disodium salts.

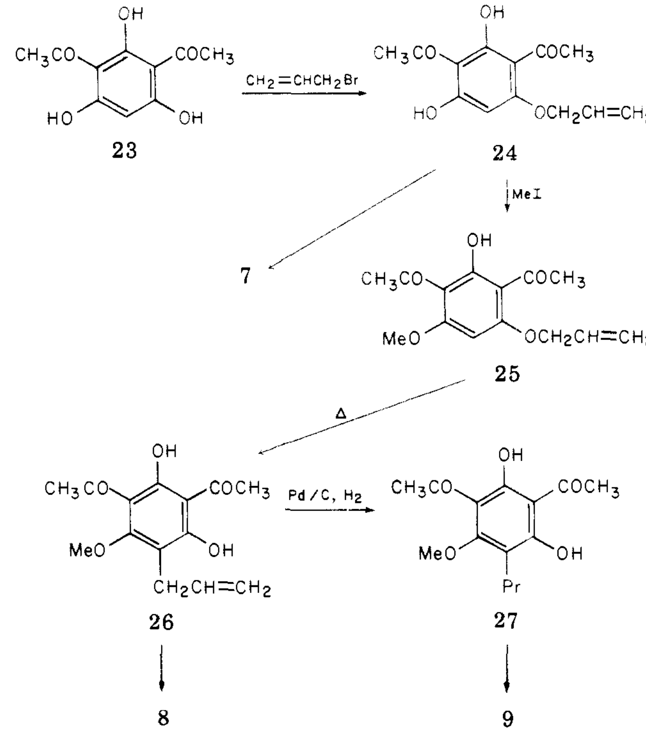
Table I



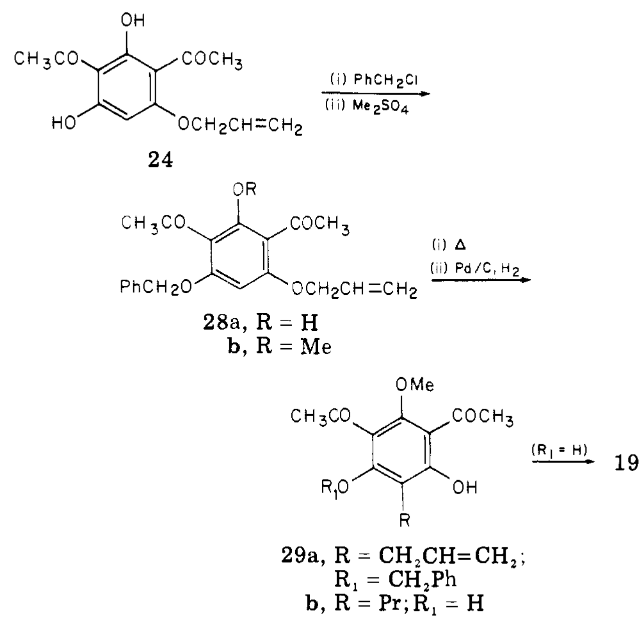
Compd no.	R1	R2	Lit. ref to precursor	Mp, °C (ester)	Mp, °C (acid)	Mol formula[a] of free acid	PCA ID_{50},[b] mg/kg	Range,[c] mg/kg	Method of prepn	% yield
I							1.2[d]			
1	OMe	H	15	203-204	262-263	$C_{15}H_8O_9 \cdot H_2O$	0.73	0.5-2.0	A	26
2	OH	H			300-302	$C_{14}H_6O_9 \cdot H_2O$	5.0	2.5-10.0	C	57
3	H	Et	16		282	$C_{16}H_{10}O_8 \cdot H_2O$	10.0	e	A (i)	<10
4	OEt	H	15	183-184	275-277	$C_{16}H_{10}O_9 \cdot H_2O$	0.38	0.25-0.5	A (i)	20
5	H	H	17			$C_{14}H_4Na_2O_8$	7.6	5-10	A (ii)	46
6	H	Cl			301 dec	$C_{14}H_5ClO_8 \cdot H_2O$[f]	>10		B	30
7	$OCH_2CH{=}CH_2$	H			250-253 dec	$C_{17}H_{10}O_9 \cdot 0.5H_2O$	0.62	0.5-1.0	A (i)	<10
8	OMe	$CH_2CH{=}CH_2$			253-259 dec	$C_{18}H_{12}O_9 \cdot H_2O$	0.22	0.2-0.25	A (i)	<10
9	OMe	Pr		154-155	268-270	$C_{18}H_{14}O_9 \cdot H_2O$	0.18	0.15-0.2	A (ii)	20

[a] All compounds were analyzed for C and H, and N and Cl where appropriate. [b] All compounds were tested by intravenous administration of their water-soluble sodium salts. The purity of the salts was checked by TLC examination, on silica gel, prior to test. [c] This is the range of doses within which the estimated ID_{50} was found to lie. [d] This is the mean value of 33 determinations for sodium cromoglycate. The SE is ±0.08. [e] Tested only at 10 mg/kg and gave 50% inhibition at this dose. [f] Cl: calcd, 10.0; found, 10.49.

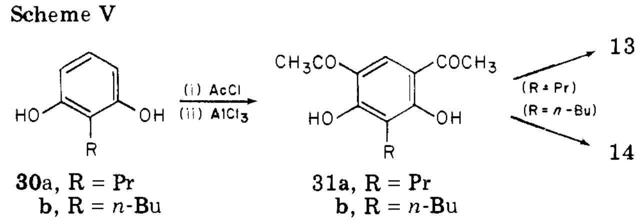
Scheme III



Scheme IV

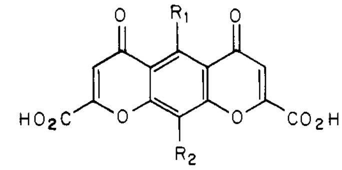


Scheme V



The diacetyldihydroxybenzene precursors for compounds **1**, **3–5**, **11**, **12**, **15**, **16**, **18**, and **20–22** were prepared by literature methods (for references, see tables), as was 2-ethyl-5-methylresorcinol[25] the starting material for compound **10**. 4-Chlororesorcinol, the precursor of compound **6**, was commercially available. The starting materials for the other compounds were prepared, using standard reaction conditions, by the routes shown in

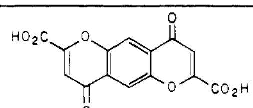
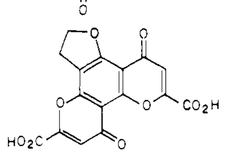
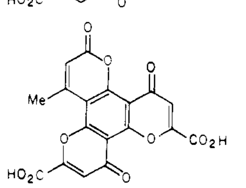
Table II



Compd no.	R_1	R_2	Lit. ref to precursor	Mp, °C (ester)	Mp, °C (acid)	Mol formula[a] of free acid	PCA ID_{50},[b] mg/kg	Range,[c] mg/kg	Method of prepn	% yield
10	Me	Et	25		312 dec	$C_{17}H_{12}O_8\cdot 0.5H_2O$	0.03	0.01-0.05	B	16
11	H	Me	18		293-294	$C_{15}H_8O_8\cdot 0.5H_2O$	0.25	0.1-0.5	A (i)	31
12	H	Et	19	189-191	315	$C_{16}H_{10}O_8$	0.1	0.1-0.5	A (ii)	57
13	H	Pr		154-156[d]	310-311	$C_{17}H_{12}O_8\cdot 0.5H_2O$	0.05	0.05-0.1	A (ii)	35
14	H	*n*-Bu		155-157	314 dec	$C_{18}H_{14}O_8$	0.05	0.05-0.075	A (ii)	60
15	H	NO_2	20	215	225-227	$C_{14}H_5NO_{10}\cdot 2H_2O$	1.6	1.0-2.5	A (ii)	<10
16	H	H	21	225-226		$C_{14}H_4Na_2O_8$	0.18	0.1-0.5	A (ii)	51
17	H	CH_2-OEt	15	171-173		$C_{17}H_{10}Na_2O_9$	0.33	0.25-0.5	A (ii)	<10
18	OMe	H	15	227-228		$C_{15}H_6Na_2O_9$	0.32	0.1-0.5	A (ii)	78
19	OMe	Pr		168-168.5	278-279 dec	$C_{18}H_{14}O_9$	0.1	*e*	A (ii)	68

[a] See Table I, footnote *a*. [b] See Table I, footnote *b*. [c] See Table I, footnote c. [d] C: calcd, 62.99; found, 62.3. [e] A dose of 1.0 mg/kg gave 100% inhibition; the other dose tested, 0.1 mg/kg, gave 50% inhibition.

Table III

Compd no.	Structure	Lit. ref to precursor	Mp, °C (ester)	Mp, °C (acid)	Mol formula[a] of free acid	PCA ID_{50},[b] mg/kg	Range,[c] mg/kg	Method of prepn	% yield
20		22	244-245	>340	$C_{14}H_6O_8\cdot 1\cdot 5H_2O$	0.68	0.5-1.0	A (ii)	44
21		23		294-296	$C_{16}H_8O_9\cdot 2H_2O$	0.35	0.1-0.5	A (i)	25
22		24		263-267 dec	$C_{18}H_8O_{10}\cdot 3H_2O$	4.24	2.5-5.0	A (i)	<10

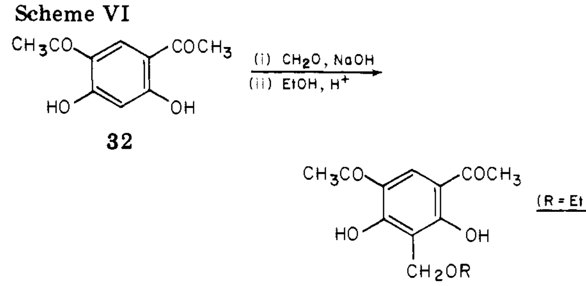
[a] See Table I, footnote *a*. [b] See Table I, footnote *b*. [c] See Table I, footnote c.

Schemes III (7-9), IV (19), V (13 and 14), and VI (17) (for details see the Experimental Section).

Structure-Activity Relationships. Of the three parent compounds tested, the linear benzodipyrans **16** and **20** were considerably more active than the angular isomer **5**. In fact, this was generally true of the two series as a whole; the linear compounds **10-20** tended to be more active than the angular series **1-9**, **21**, and **22**. It is, however, not possible to be too certain about this relationship between activity and the geometry of the ring systems, since relatively few direct isomers were studied.

It is interesting to note the different influences of alkoxy and alkyl groups upon the activity of the two series. In the angular series the introduction of an alkoxy group ortho to one of the pyrone C=O groups resulted in a marked increase in activity, **1**, **4**, and **7**, while the presence of an alkyl group on its own had little effect on activity, **3**. This contrasted with the linear series where three of the alkyl-substituted analogues **10**, **13**, and **14** were the most active of all the compounds studied, while in the one

Scheme VI



32

33a, R = H
b, R = Et

17

compound tested, **18**, the alkoxy group produced no enhancement of activity.

Considering the lack of influence of the alkyl group in **3**, over the parent **5**, it was surprising to note that in the angular series the combination of an alkoxy and an alkyl group in the same molecule, **8** and **9**, resulted in an increase in potency over the alkoxy-substituted compound **1**.

Compound **21** can also be considered a further example of this phenomenon if one regards the furano ring as representing an alkoxy and an alkyl substitutent on the angular benzodipyran ring system. The introduction of electron-withdrawing substituents into either the angular, **6**, or the linear, **15**, series resulted in loss of activity compared to the parent compounds **5** and **16**, respectively.

Experimental Section

Biological Test Procedure. The passive cutaneous anaphylaxis (PCA) test in rats was used to assess the antiallergic activity of the compounds discussed. Serum containing homocytotropic reaginic antibody to *Nippostrongylus braziliensis* was collected from rats repeatedly injected with *N. braziliensis* larvae. Antigen was prepared from *N. braziliensis* worms using the method of Goose and Blair.[13]

Antisera were titrated in female Sprague–Dawley rats weighing 80–100 g. Using groups of five rats, 0.1 ml of increasing twofold dilutions of antiserum was injected intradermally into the flank of each rat. After a latent period of 24 h, rats were challenged intravenously with worm antigen (25 mg/kg of protein) in the presence of Evans blue dye (25 mg/kg). Rats were killed 30 min after challenge, the skin was reflected, and PCA reactions were graded according to the area of extravasation of dye as follows: 0–4 mm reaction diameter = grade 0; 5–9 mm = grade 1; 10–14 mm = grade 2; 15–20 mm = grade 3; and >20 mm = grade 4.

Compounds were tested using PCA reactions induced by the greatest dilution of antiserum giving grade 4 reactions in each control rat tested. This dilution of antiserum varied from 1 in 8 to 1 in 32 in these experiments. Compounds were dissolved in saline and varying doses were injected intravenously to groups of five rats with antigen challenge. Inhibition of PCA reactions was calculated using the following formula.

$$\% \text{ inhibition} = 100 - \frac{\text{(mean score of treated group)}}{\text{(mean score of control group)}} \times 100$$

The percentage inhibition of PCA was plotted against dose on a logarithmic scale and the drug dose giving 50% inhibition of PCA (ID_{50}) was interpolated from the graph. DSCG was used as a standard compound on each test day and its mean ID_{50} with statistical limits over the whole series of experiments is shown in Table I.

Melting points are uncorrected. Elemental analyses for the compounds were within ±0.4% of the theoretical value, unless otherwise stated. The ir, mass, and NMR spectra of the compounds were consistent with the assigned structures.

Method A. 5-Methoxy-4,10-dioxo-4*H*,10*H*-benzo[1,2-*b*:3,-4-*b'*]dipyran-2,8-dicarboxylic Acid (1). To a stirred ice-cooled solution of Na (3.04 g) in EtOH (40 ml) was added a mixture of 2,4-diacetyl-5-methoxyresorcinol[15] (3.7 g) and diethyl oxalate (12.05 g) in EtOH (20 ml) and Et_2O (50 ml). The mixture was stirred and heated under reflux for 4 h and then cooled to room temperature. Et_2O (100 ml) and H_2O (200 ml) were then added and the aqueous layer was separated, acidified with concentrated HCl, and extracted wih EtOAc (2 × 100 ml). After drying over $MgSO_4$, the EtOAc solution was evaporated to dryness to leave a brown oil. This oil was dissolved in boiling EtOH (100 ml) containing concentrated HCl (0.5 ml) and the solution (A) was heated under reflux for 30 min. At this stage two alternative workup procedures were used to give either **1** directly or its diethyl ester.

(i) The solution (A) was evaporated to dryness and the remaining oil was triturated with Et_2O to give a brown solid. This solid was added to an aqueous solution of $NaHCO_3$ and the mixture was heated until the solid completely dissolved. The solution was cooled and acidified with concentrated HCl to give 0.96 g of **1** as a light brown solid.

(ii) The solution (A) was concentrated to small volume (30 ml) and left overnight at room temperature. The diethyl ester of **1**, which crystallized out, was filtered off: yield 1.3 g. The diester was dissolved in boiling aqueous EtOH and $NaHCO_3$ (1 g) was added. Heating was continued until TLC indicated that hydrolysis of the diester was complete. The remaining solution was cooled and acidified with concentrated HCl to give 0.9 g of **1** as a light brown solid.

When the disodium salts of **5** and **16–18** were prepared directly from the corresponding diesters, the following general procedure was used. The diethyl ester of **5** (1.6 g) was suspended in EtOH (50 ml) and aqueous NaOH (8.7 ml of 1.042 N) was added. The yellow suspension was heated at 100° for 10 min. After cooling, the insoluble solid was filtered off, washed with hot EtOH, and crystallized by dissolving in H_2O and then adding an equal volume of EtOH to give the disodium salt of **5**: yield 1.0 g.

Method B. 10-Ethyl-5-methyl-4,6-dioxo-4*H*,6*H*-benzo-[1,2-*b*:5,4-*b'*]dipyran-2,8-dicarboxylic Acid (10). A solution of VIIb (3.04 g) and dimethyl acetylenedicarboxylate (6.5 g) in dioxane (10 ml) was treated with 3 drops of a 40% aqueous solution of benzyltrimethylammonium hydroxide. The mixture was heated on a steam bath for 0.25 h, cooled and treated with 25% aqueous NaOH (25 ml), and reheated on a steam bath for 1 h. The mixture was cooled, washed with Et_2O to remove dioxane, acidified with 5 N H_2SO_4 solution, and extracted wih Et_2O. Evaporation of the ethereal extract left an orange solid (7.7 g). This orange solid (3 g) was dissolved in $ClSO_3H$ at room temperature, with stirring, then heated at 80° for 0.5 h, cooled, and cautiously poured on to ice (200 g). A precipitate was formed which was allowed to settle under gravity and was then filtered off, briefly boiled with dilute aqueous EtOH, and reprecipitated from bicarbonate solution to give **10** as a pale orange solid: yield 1.2 g.

Method C. 5-Hydroxy-4,10-dioxo-4*H*,10*H*-benzo[1,2-*b*:3,-4-*b'*]dipyran-2,8-dicarboxylic Acid (2). A solution of **1** (0.55 g) in HBr-AcOH (7 ml of 45%) and glacial AcOH (7ml) was heated under reflux for 1.5 h during which time a solid separated. The mixture was poured into H_2O (100 ml) and the solid was filtered off and washed thoroughly with H_2O to give **2** as an off-white solid: yield 0.3 g.

The novel precursors described in Schemes III–VI were prepared by the following procedures.

2,4-Diacetyl-5-allyloxyresorcinol (24). A mixture of **23**[15] (10.5 g), allyl bromide (6.05 g), and anhydrous K_2CO_3 (6.9 g) in dry Me_2CO (200 ml) was refluxed for 2 days. After cooling, the solid was filtered off and the solution was evaporated to dryness, leaving a solid. This solid was crystallized from EtOH to give **24** as colorless needles: yield 3.32 g; mp 111–112°.

2,4-Diacetyl-6-allyl-5-methoxyresorcinol (26). A mixture of **24** (15.5 g), anhydrous K_2CO_3 (8.6 g), MeI (10 ml), and dry Me_2CO (200 ml) was refluxed for 16 h, concentrated in vacuo, and acidified with dilute HCl. Extraction with Et_2O and subsequent removal of the solvent gave **25** as a red oil (17 g) distilling at 148–162° (0.3 mm) (not characterized). The distillate and tetralin were refluxed together for 3.5 h, cooled, poured into 2 N NaOH (500 ml), and washed with C_6H_6. Repeated extraction of the aqueous alkaline phase with EtOAc and evaporation of the combined EtOAc portions gave a solid which crystallized from aqueous EtOH to afford **26** as fibrous needles: yield 4.5 g; mp 84.5–85°.

2,4-Diacetyl-5-methoxy-6-propylresorcinol (27). Compound **26** (1.3 g) in EtOH was hydrogenated over 5% Pd/C at 3 atm for 2 h. Filtration of the mixture and evaporation afforded an oil, which crystallized from aqueous EtOH to give **27** as needles: yield 0.8 g; mp 48–49°.

2,6-Diacetyl-3-allyloxy-5-benzyloxyphenol (28a). A mixture of **24** (13.5 g), anhydrous K_2CO_3 (7.5 g), benzyl chloride (13 ml), KI (0.5 g), and dry Me_2CO (60 ml) was stirred and refluxed for 43 h. Concentration of the mixture and acidification with dilute HCl gave an oil which was taken into Et_2O. Repeated extraction with 2 N NaOH and subsequent acidification gave a solid, which was recrystallized from aqueous EtOH to afford **28a** as pale yellow needles: yield 6 g; mp 92°.

3-Acetyl-4-allyloxy-6-benzyloxy-2-methoxyacetophenone (28b). Compound **28a** (6 g), anhydrous K_2CO_3 (6.5 g), Me_2SO_4 (7.5 ml), and dry Me_2CO (150 ml) were stirred and refluxed for 16 h. Acidification followed by extraction with Et_2O afforded a solid, which crystallized from aqueous EtOH (charcoal) to give **28b** as prisms: yield 4 g; mp 77–78°.

4,6-Diacetyl-5-methoxy-2-propylresorcinol (29b). Compound **28b** (6.6 g) was refluxed in tetralin (15 ml) under N_2 for 4 h, diluted with petroleum ether, and extracted repeatedly with

2 N NaOH. Acidification of the aqueous extracts gave a red oil, which was chromatographed on silica gel with Et_2O–petroleum ether (1:7) to yield **29a** (2.4 g) as an oil (not characterized). This oil was hydrogenated in ethanol containing HCl (1 ml) at 3 atm in the presence of 5% Pd/C for 1 h. Filtration and evaporation gave an oil, which was extracted repeatedly with hot petroleum ether to leave a tar. The extracts were evaporated, and the residue was distilled at 150–170° (0.6 mm) to give a viscous oil which crystallized from aqueous EtOH yielding **29b** (1.1 g) as needles: mp 80°.

4,6-Diacetyl-2-hydroxymethylresorcinol (33a). Formaldehyde (40%, 0.3 ml) was added to a solution of **32** (1 g) in aqueous NaOH (1%, 27 ml) at room temperature. After 5 min the solution was acidified and extracted with Et_2O. The dried Et_2O extracts on evaporation gave a product which was purified by chromatography on a silica gel column using Et_2O as eluent to give 0.9 g of **33a**: mp 150–151°.

4,6-Diacetyl-2-ethoxymethylresorcinol (33b). A mixture of **33a** (2.0 g), concentrated H_2SO_4 (5 drops), and dry EtOH (100 ml) was refluxed for 2 h. On cooling, **33b** crystallized out as long needles (1.9 g): mp 163–165°.

4,6-Diacetyl-2-propylresorcinol (31a) and 4,6-Diacetyl-2-butylresorcinol (31b). These two compounds were prepared by analogous procedures. Thus the diacetate derivatives of 2-propyl- and 2-butylresorcinol[25] were prepared using AcCl–pyridine under standard O-acylation conditions. The esters were then subjected, in the absence of a solvent, to Fries rearrangement with $AlCl_3$ (for 1 h at 130–150°) to give **31a** and **31b** on quenching with ice-cold HCl. Neither of the diacetates nor **31a** was characterized but **31b** crystallized as colorless needles from EtOH: mp 61–64°.

pK_a Determination. Dissociation constants were obtained for two of the acidic compounds by potentiometric titrations in a series of concentrations of aqueous 2-methoxyethanol[26] at 25°. The overlapping titration curves for the two carboxy groups were separated mathematically to give pK_{a_1} and pK_{a_2}.[27] The spread of values in the determination of pK_{a_1} and pK_{a_2} was $\leq\pm0.1$ pH units for each compound. Curves were plotted to relate pK_{a_1} and pK_{a_2} to zero organic solvent content to yield the following values of pK_{a_1} and pK_{a_2} in water: **1**, $pK_{a_1} = 1.3$, pK_{a_2} 2.4; **12**, $pK_{a_1} = 1.3$, $pK_{a_2} = 2.35$.

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References and Notes

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