Synthesis of 2-Hydroxyquinic and 2-Hydroxyepiquinic Acids from Shikimic Acid[1]

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Abstract: 1,2-Anhydro-(2*S*)-2-hydroxyquinic (epoxyshikimic) acid (**16**) has been prepared by treating (2*R*)-2-bromoquinic acid (**14**) or (2*R*)-2-bromoquinolactone (**15**) with aqueous $Ba(OH)_2$ at 0°. Oxirane ring cleavage of **16** with $Ba(OH)_2$ at 100° gave (2*R*)-2-hydroxyquinic acid (**4**) and (2*S*)-hydroxyepiquinic acid (**12**), whereas cleavage with glacial acetic acid gave 1,4-anhydro-(2*S*)-hydroxyquinic acid (**32**). Treatment of 3,4,5-tri-*O*-acetyl-1,2-anhydro-(2*S*)-2-hydroxyquinic acid (**18**) with glacial acetic acid followed by saponification afforded (2*S*)-2-hydroxyquinic acid (**3**). The course of oxirane cleavage of **16** and **18** with glacial acetic acid, aqueous acetic acid, and perchloric and *p*-toluenesulfonic acids in dioxane or acetone was studied by vpc analysis of the ratio of **3**:**4**:**12**:**32**. The methyl esters of **16** and **18** did not undergo oxirane ring cleavage with the above reagents. It was postulated that carboxyl group participation, probably by α-lactone and β-lactone formation, may be one of the mechanisms involved in oxirane ring cleavage of **16** and **18**. (2*R*)-2-Hydroxyepiquinic acid (**10**) was prepared from the previously synthesized methyl 5-acetyl-3,4-isopropylidene-(2*R*)-2-hydroxyepiquinate (**9**), and by hydroxylation of methyl shikimate (**7**) with OsO_4.

In synthetic studies of potential intermediates in the enzymic conversion of 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (**1**) to 5-dehydroquinic acid (**2**)[4] it was of interest to synthesize the 2-hydroxy derivatives **3** and **4** of quinic acid (**5**) and their 2-*O*-phosphate esters. Shikimic acid (**6**) appeared as a promising starting material, since configurations are identical at C-3, -4, and -5 of 2-hydroxyquinic and shikimic acids, and only hydroxylation of the double bond of **6** is needed to prepare **3** and **4**. Methyl 5-acetyl-3,4-*O*-isopropylideneshikimate (**8**) was hydroxylated with permanganate by Fischer and Dangschat[5] to yield a dihydroxy derivative **9**. The free pentahydroxy compound was not isolated, and the stereochemistry of the reaction was not studied. However, attack by permanganate ion on the double bond of **8** would be expected to result in cis hydroxylation of the less hindered side of the molecule, *i.e.*, opposite the isopropylidene group, to give **9**. Acid and then alkaline hydrolysis of **9** gave the pentahydroxy acid **10**, and the same compound was obtained in the absence of the bulky isopropylidene group by the hydroxylation of methyl shikimate (**7**) with OsO_4, a reagent also known to give cis hydroxylation.[6] The configuration assigned to **10** of a (2*R*)-2-hydroxy derivative of epiquinic acid (**11**) was supported by the method of its synthesis, and confirmed by its nmr spectra, as discussed below. The availability of **10** facilitated structural studies of **3**, **4**, and **12** synthesized as described in the Experimental Section.[7]

Synthesis

Many attempts to prepare **3** or **4** by altering the stereochemistry of attack at the double bond of shikimic acid or its derivatives failed, owing to poor reactivity of the double bond in addition reactions.[8] We therefore prepared 1,2-epoxyshikimic acid (**16**) from shikimic acid (**6**) *via* the 1,2-dibromide (**13**) and (2*R*)-2-bromoquinic acid (**14**).[13,14] The structures of **13** and **15** established by the work of Grewe and Lorenzen[14] were confirmed by a more recent nmr study of Haslam and Turner[15] and by the present investigation. Treatment of **14** or its lactone **15** with 2 equiv of $Ba(OH)_2$ under controlled conditions of pH and temperature afforded **16** in good yield.[16] Either a 1,2- or a 2,3-epoxide could

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(2) Career Investigator Fellow of the American Heart Association, 1969–1971.

(3) Career Investigator of the American Heart Association.

(4) M. Adlersberg and D. B. Sprinson, *Biochemistry*, 3, 1855 (1964).

(5) H. O. L. Fischer and G. Dangschat, *Helv. Chim. Acta*, 20, 705 (1937).

(6) R. Criegee and P. Becher, *Chem. Ber.*, 90, 2516 (1957).

(7) The majority of the compounds of this study are C-2 substituted derivatives of quinic acid (**5**) and of epiquinic acid (**11**). Since the configurations of the hydroxyl groups at C-3, C-4, and C-5 remain unchanged, we have adopted a nomenclature based on the configuration of C-1 and C-2. Thus **3**, **4**, **10**, **12**, and **14** will be called, respectively, (2*S*)-2-hydroxyquinic, (2*R*)-2-hydroxyquinic, (2*R*)-2-hydroxyepiquinic, (2*S*)-2-hydroxyepiquinic, and (2*R*)-2-bromoquinic acid; **16** is 1,2-anhydro-(2*S*)-2-hydroxyquinic acid, but will be referred to as "epoxishikimic acid." A nomenclature based on *R* and *S* designation of each assymmetric center would be: **3**, (1*R*,2*S*,3*S*,4*S*,5*R*)-1,2,3,4,5-pentahydroxycyclohexane-1-carboxylic acid; **4**, (1*R*,2*R*,3*S*,4*S*,5*R*)-1,2,3,4,5-pentahydroxycyclohexane-1-carboxylic acid; **10**, (1*S*,2*R*,3*S*,4*S*,5*R*)-1,2,3,4,5-pentahydroxycyclohexane-1-carboxylic acid; **12**, (1*S*,2*S*,3*S*,4*S*,5*R*)-1,2,3,4,5-pentahydroxycyclohexane-1-carboxylic acid; **16**, (1*R*,2*S*,3*S*,4*S*,5*R*)-1,2-anhydro-3,4,5-trihydroxycyclohexane-1-carboxylic acid; **32**, (1*R*,2*S*,3*S*,4*S*,5*R*)-1,4-anhydro-2,3,5-trihydroxycyclohexane-1-carboxylic acid.

(8) Examples of reactions which failed are: cis hydroxylation of **8** with iodine and silver acetate in moist acetic acid[9] which was expected to proceed with a stereochemistry opposite to that obtained with permanganate; trans hydroxylation (*via* epoxidation) of **7** with hydrogen peroxide and sodium tungstate;[10] epoxidation of **8** with 3-chloroperbenzoic acid;[11] epoxidation of **8** or tri-*O*-acetylshikimic acid with 4-nitroperbenzoic acid.[12] Attempts to prepare 2-hydroxyquinic acid derivatives by displacement of bromine on C-2 of **37** and **38** also failed, and are summarized at the end of the Experimental Section.

(9) S. Winstein and R. E. Buckles, *J. Amer. Chem. Soc.*, 64, 2787 (1942); R. B. Woodward and F. V. Brutcher Jr., *ibid.*, 80, 209 (1958).

(10) G. B. Payne and P. H. Williams, *J. Org. Chem.*, 24, 54 (1959).

(11) N. N. Schwartz and J. H. Blumbergs, *ibid.*, 29, 1976 (1964).

(12) R. Hollands, D. Becher, A. Gaudemer, and J. Polonsky, *Tetrahedron*, 24, 1633 (1968).

(13) J. F. Eykman, *Chem. Ber.*, 24, 1278 (1891).

(14) G. Grewe and N. Lorenzen, *ibid.*, 86, 928 (1953).

(15) E. Haslam and M. J. Turner, *J. Chem. Soc.*, 1496 (1971).

(16) Eykman[13] prepared (2*R*)-2-bromoquinolactone (**15**) by treating the dibromide **13** with water. He also described the preparation of an optically active dihydroxyshikimic acid by treating **15** with aqueous $Ba(OH)_2$. However, in our hands, treating **15** with base afforded only the epoxy acid **16**.

Table I. Coupling Constants (Hz) of Epoxyshikimic Acid, 2-Substituted Quinolactone and Epiquinolactone Derivatives, and 1,4-Anhydro Compound **36**[a]

Compd	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6e}$	$J_{5,6a}$	$J_{6e,6a}$	W coupling
16[b]	4.0	5.0	8.5	5.0	7.6	−15.0	
39	10.5	5.0	4.5	2.5[c]	2.5[c]		
25[d]	9.5	5.2	4.5	6.0	0	−12.3	$J_{4,6e}$ 1.0
27[e]	4.5	4.5	5.0	6.0	0	−12.0	$J_{2,6e}$ 1.5[f]
20	0	1.0	8.5	7.5	10.5	−12.0	
31[g]	6.5	1.5	8.5	7.5	11.0[h]	−12.5	$J_{2,6e}$, 1.5[i]
36	6.0	0	5.0	10.2	3.7	−13.8	

[a] Assignments made from analysis of spin interactions at 100 MHz and, except for **39**, at 220 MHz. [b] Small coupling observed in H-6e resonance, $J \sim 0.8$ Hz, may be due to interaction with H-2. [c] Average of $J_{5,6e}$ and $J_{5,6a}$ since H-6a and H-6e are magnetically equivalent. [d] Assignments of H-4 and H-5 confirmed by spin decoupling of 100-MHz spectrum at δ 3.19 and 4.84. [e] Assignments of H-4 and H-5 confirmed by spin decoupling of 100-MHz spectrum at δ 3.21 and 4.90. [f] Assignment confirmed by spin decoupling of 100-MHz spectrum at δ 5.54 and 3.21. [g] Assignments of H-2, H-3, H-4, and H-5 confirmed by spin decoupling of 100-MHz spectrum at δ 2.44, 4.85, 5.11, and 6.27. [h] Obtained from analysis of H-5 at δ 5.11 since H-6a was obscured. [i] Assignment confirmed by spin decoupling of 100-MHz spectrum at δ 6.27 and 2.44.

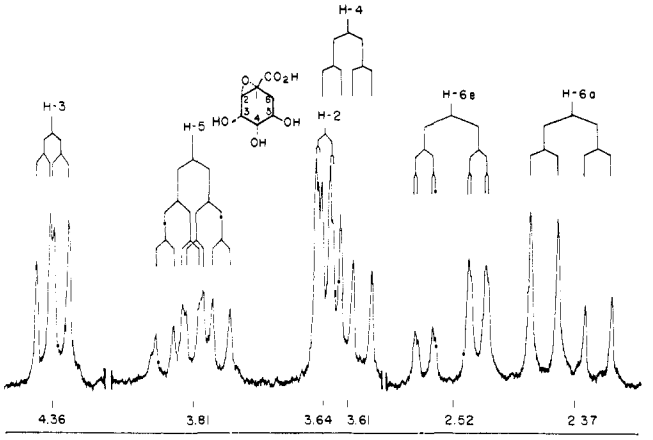
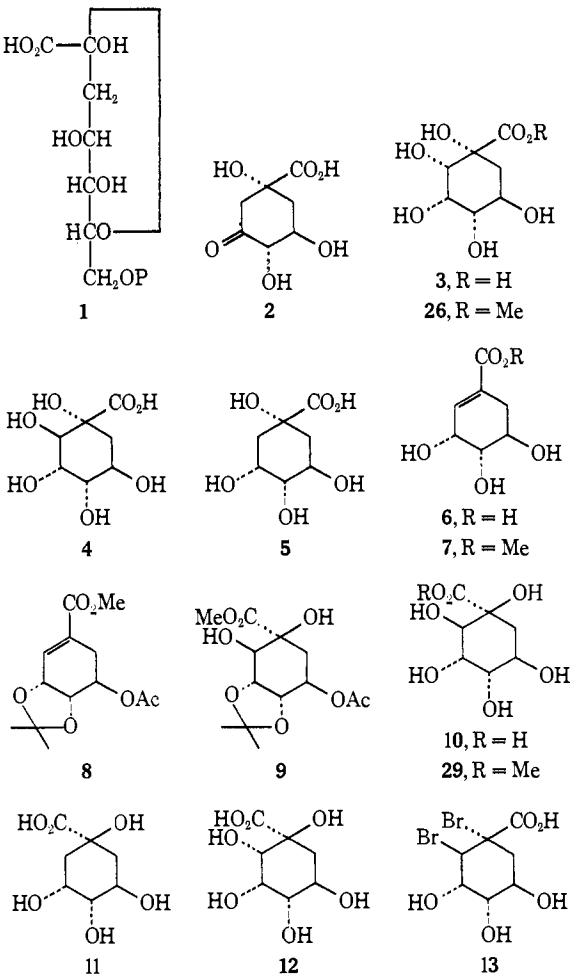


Figure 1. 220-MHz nmr spectrum of 1,2-anhydro-(2*S*)-2-hydroxyquinic acid (**16**) in ^2H_2O.

be formed by treating **14** with base since the hydroxyl groups at both C-1 and C-3 are in a trans diaxial relationship to the bromine at C-2. A 1,2-epoxide structure for **16** was in accord with its consumption of 2 equiv of periodate/mol, and was established by analysis of its nmr spectrum. The configurations of C-1 and C-2 were assigned on the reasonable assumption of SN2 displacement of bromine by the hydroxyl group at C-1.

A 220-MHz spectrum of epoxyshikimic acid in ^2H_2O may be seen in Figure 1, and the coupling constants are in Table I. The resonances of the C-6 methylene protons and H-5 verge on a second-order ABX pattern but were analyzed by first-order rules and errors were judged to be small. Although the absorption of H-2 overlapped that of H-4, it was possible to obtain their chemical shifts and their coupling constants with adjacent protons by analysis of the spectrum (Figure 1). These absorptions were clearly resolved in a 100-MHz spectrum of **17** in a solution of ^2H_2O, acetone-d_6, and pyridine-d_5, and gave coupling constants similar to those measured in D_2O. However, H-6 methylene protons appeared as a doublet, $J_{5,6ae} = 5.2$ (average of $J_{5,6a}$ and $J_{5,6e}$), owing to magnetic equivalence of H-6a and H-6e in this solvent. Assignment of **16** as a 1,2-epoxide is in accord with the chemical shift of H-2, δ 3.64. In glycidic acid the cis β proton has δ 2.93, and alkylation of the β position would shift this value downfield by ~0.5 ppm to δ 3.48.[17] Furthermore, H-3 (δ 4.35) is too deshielded for an oxirane ring proton of a 2,3-epoxide. In 1-methylcyclohexene epoxide δ 2.45 was found for the oxirane proton.[18] Epoxyshikimic acid may adopt any one of two semichair and two boat conformations of which **16a** and **b** are energetically favored. Comparison of dihedral angles (Table II) obtained from Dreiding models of **16a** and **16b** with those calculated from the coupling constants in Table I shows that **a** is the preferred conformation for **16** in water.



(17) L. M. Jackman and S. Sternhell, "Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed, Pergamon Press, Elmsford, N. Y., 1969, p 228.

(18) D. Farcasiu, C. Kascheres, and L. H. Schwartz, *J. Amer. Chem. Soc.*, **94**, 180 (1972).

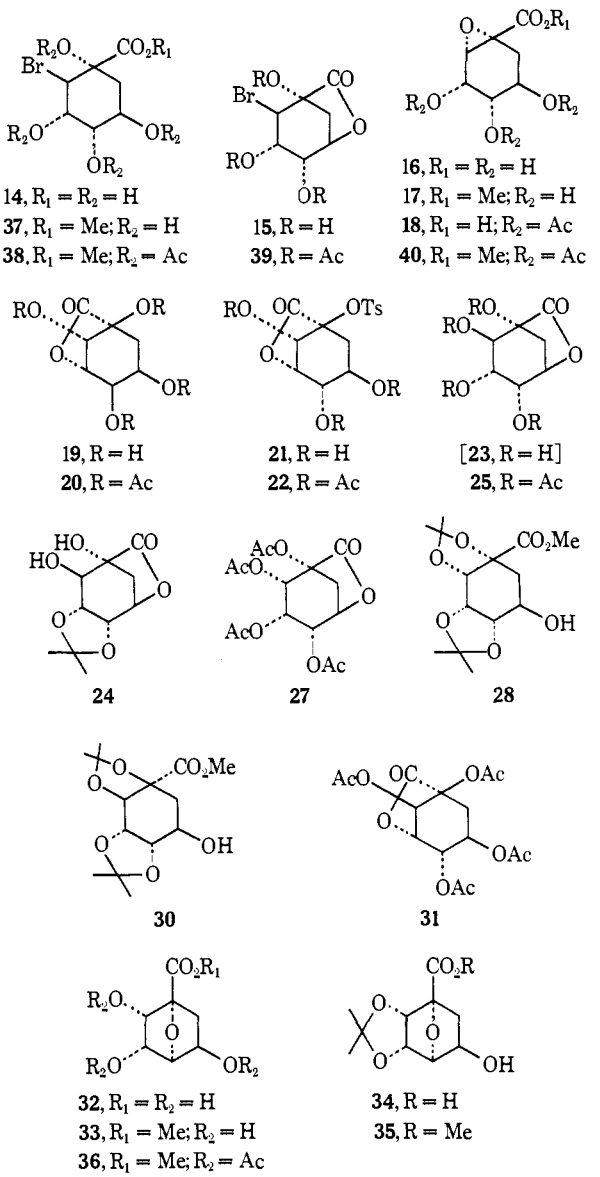
The two unknown 2-hydroxyquinic acids **3** and **4** and the unknown (2*S*)-2-hydroxyepiquinic acid **12** were prepared by cleavage of the oxirane ring of **16** or its tri-*O*-acetate under controlled conditions. The structures of the products were assigned mainly by analysis of the nmr spectra of their γ-lactone tetra-*O*-acetates or other derivatives (as discussed below). The course of oxirane ring opening reactions was followed by vpc of trimethylsilyl drivatives.

Treating the epoxy acid **16** with aqueous $Ba(OH)_2$ gave the crystalline γ-lactone of **12** (**19**) as a major product. Similarly, *p*-toluenesulfonic acid monohydrate in dioxane gave a high yield of the same lactone as 1-*O*-*p*-toluenesulfonate (**21**). Examination by vpc of the crude syrup from the reaction of **16** with $Ba(OH)_2$ showed the presence of a minor component in about one-half the concentration of **19**. A sample of the residue resulting from removal of a portion of **19** by crystallization was treated with acetic anhydride, and the product was shown by nmr to contain the γ-lactone tetra-*O*-acetate of **4** (**25**). This residue was converted to a mixture of acetonides from which the 3,4-*O*-isopropylidene-5-lactone of **4** (**24**) was isolated as a crystalline product.

In contrast to the above results, reaction of **16** with glacial acetic acid yielded 1,4-anhydro-(2*S*)-2-hydroxyquinic acid (**32**) which was isolated as a monohydrate. The structure assigned to **32** is in accord with its ability to form a monoacetonide (**34**, **35**) but not a lactone, consumption of 1 equiv of periodate, and the nmr spectra, which will be discussed below, of its methyl ester (**33**), methyl ester tri-*O*-acetate (**36**), and methyl ester acetonide (**35**).

Oxirane ring cleavage of 3,4,5-tri-*O*-acetylepoxyshikimic acid (**18**) with glacial acetic acid gave (2*S*)-2-hydroxyquinic acid (**3**), in high yield, together with small amounts of the lactones of **4** and **12** which were detected by vpc. The methyl ester of **3** (**26**) formed a diacetonide (**28**) which was different from the diacetonide (**30**) obtained from the methyl ester of **10** (**29**). Since the two pairs of cis hydroxyl groups in **10** are in an anti relationship, those of **3** must be syn, in agreement with the structure derived from the nmr spectra.

Structure Assignments of Ring Cleavage Products of Epoxyshikimic Acid by Nmr Spectroscopy. The bromolactone (**15**), the product of permanganate oxidation (**10**), and the products of oxirane cleavage (**3**, **19**, **23**) were converted to their γ-lactone acetates **39**, **31**, **27**, **20**, and **25**, respectively. Determination of configuration at C-1 and C-2 by nmr was greatly facilitated by the fixed conformation in the cyclohexane ring owing to the γ-lactone structure. The acetates also showed greater separation of ring proton resonances than their corresponding parent compounds. Nmr spectra of the lactone tetra-*O*-acetates **20**, **25**, **27**, and **31** at 220 MHz are shown in Figures 2–5, in which resonances of acetate protons, δ 1.98–2.25, are deleted. Coupling constants, derived from analysis of 100- and 220-MHz spectra, and decoupling experiments which confirmed assignments of several individual protons are recorded in Table I. The configurations of C-1 and C-2 were assigned by calculating dihedral angles from coupling constants[19,20] and contrasting the calculated values



with those obtained from appropriate models (Table II). The latter were constructed by assuming that configurations of C-3, C-4, and C-5 were the same as in shikimic acid, and that the double bond was replaced by a diol. It is clear that the configuration at C-1 will direct γ-lactone formation to the hydroxyl at C-5 or at C-3, *i.e.*, in the quinic acid series lactonization will occur at C-5 while in the epiquinic acid series lactonization will occur at C-3. Although δ-lactone formation is theoretically possible in the epiquinic acids, the ir spectra of **20** and **31** show that they are γ-lactones. Since the hydroxyls at C-3 and C-5 are trans to each other the cyclohexane ring in the two series of lactones will be fused in one of two unique chair conformations, as shown in the structures of Figures 2–5. Owing to the marked difference in conformation of ring protons in the two series, the quinolactones can be distinguished from epiquinolactones by nmr, thus establishing configuration at C-1. Configuration at C-2 was determined by observing the dihedral angle between H-2 and H-3 with H-2 held in either an axial or equatorial conformation, and comparing the observed value with the angle calculated from nmr spectra.

The assignment at C-1 of quinic acid configuration to **25**, **27**, and **39** and of epiquinic acid configuration to

(19) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).
(20) R. J. Abraham, L. D. Hall, L. Hough, and K. A. McLauchlan, *J. Chem. Soc.*, 3699 (1962).

Table II. Dihedral Angles of Epoxyshikimic Acid, 2-Substituted Quinolactone and Epiquinolactone Derivatives, and 1,4-Anhydro Compound **36**[a]

Model or compound	$\Phi_{2,3}$	$\Phi_{3,4}$	$\Phi_{4,5}$	$\Phi_{5,6e}$	$\Phi_{5,6a}$
16a, half-chair form	19	54	173	55	176
16b, boat form	85	55	120	56	173
16	45 (47)	39 (41)	164 (157)	39 (41)	156 (151)
1,4-Anhydro model, **36**	0	90	30	0	120
36	27 (35)	80 (81)	39 (41)	~0[b]	~130 (~129)
Quinolactone H-2e	50	55	55	40	80
Quinolactone H-2a	170	55	55	40	80
27	41 (44)	41 (44)	39 (41)	31 (35)	80 (81)
25	~180[b] (~166)	36 (40)	42 (44)	31 (35)	80 (81)
39	~180 (~180)[b]	39 (41)	41 (44)		
Epiquinolactone H-2e	40	70	170	50	170
Epiquinolactone H-2a	80	70	170	50	170
31	27 (31)	63 (64)	164 (157)	17 (24)[c]	~180 (~180)[b]
20	80 (81)	67 (68)	164 (157)	17 (24)[c]	~180 (~180)[b]

[a] Dihedral angles ($\Phi°$) measured on Dreiding models, or calculated by Karplus equation[19] with original and (in parentheses) modified parameters.[20] [b] Observed coupling constant gave $(J + 0.28)/J_0 = \cos^2\Phi > 1.0$, and the dihedral angle was estimated as 0 or 180°. [c] Williams and Bhacca[22] found $J_{ae} = 5.5 \pm 1.0$ Hz for vicinal coupling of undistorted cyclohexane ring protons ($\Phi = 60°$), one of which is geminal to an equatorial acetoxyl. This range of coupling constant yields dihedral angles of 41 (44)[a] to 26 (31)°.[a]

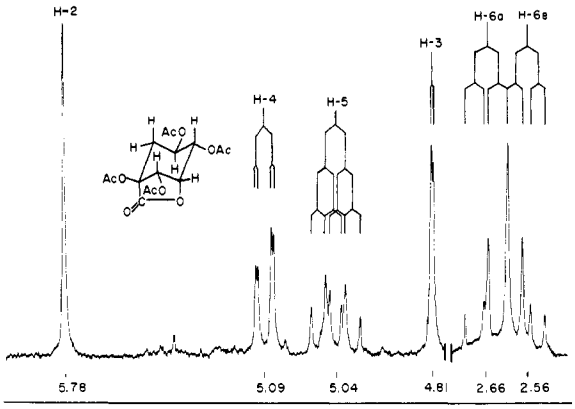


Figure 2. 220-MHz nmr spectrum of 1,2,4,5-tetra-*O*-acetyl-(2*S*)-2-hydroxyepiquino-3-lactone (**20**) in $CDCl_3$.

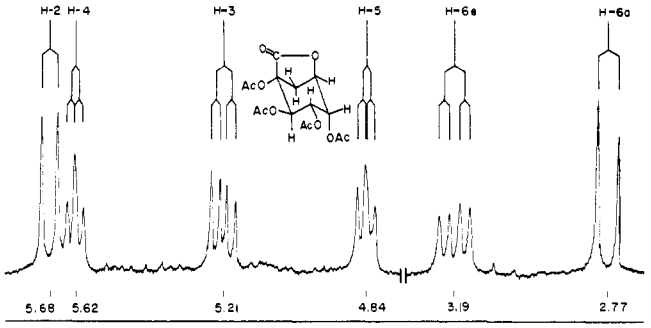


Figure 3. 220-MHz nmr spectrum of 1,2,3,4-tetra-*O*-acetyl-(2*R*)-2-hydroxyquino-5-lactone (**25**) in $CDCl_3$.

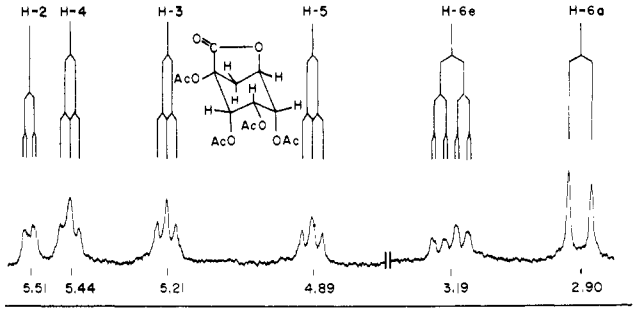


Figure 4. 220-MHz nmr spectrum of 1,2,3,4-tetra-*O*-acetyl-(2*S*)-2-hydroxyquino-5-lactone (**27**) in $CDCl_3$.

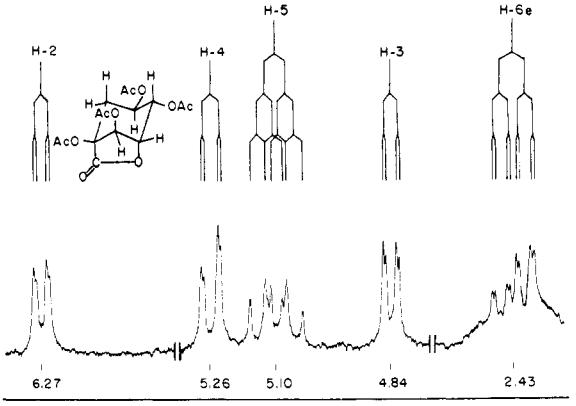


Figure 5. 220-MHz nmr spectrum of 1,2,4,5-tetra-*O*-acetyl-(2*R*)-2-hydroxyepiquino-3-lactone (**31**) in $CDCl_3$.

20 and **31** is clear from a comparison of dihedral angles measured in models with angles calculated from coupling constants (Table II). Similarly, H-2 is observed to be axial in **20**, **25**, and **39**, and equatorial in **27** and **31**. Assignments of H-2 as equatorial in **27** and **31** are further supported by observation of W couplings, $J_{2,6e} = 1.5$ Hz, which were confirmed by decoupling (Table I). A small coupling $J_{4,6e} = 1.0$ in **25** is probably also the result of W coupling. It may be observed from Table II that dihedral angles between cyclohexane ring protons in the lactones deviated from 60 and 180° owing to considerable strain which was introduced into the cyclohexane ring by the γ-lactone bridge.[21] The distortion of the cyclohexane chair conformation (Table I) in **25** and **27** was shown by $J_{5,6a} = 0$, in **20** by $J_{2,3} = 0$ and the small $J_{3,4}$, and in **31** by the small $J_{3,4}$.

In **31** the H-6a proton was shifted upfield and obscured by resonances of acetate protons. Hence, the magnitude of coupling of H-6a with H-5 was determined only by analysis of H-5 resonance. The low value calculated for $\Phi_{5,6e}$ in **20** and **31** is due to the higher than expected coupling constant $J_{5,6e}$ which can probably be accounted for by the orientation effect of

(21) E. Dunkelbaum and J. Klein, *Tetrahedron Lett.*, 55 (1968).

Table III. Cleavage Products of Epoxyshikimic Acid (16) and Its Derivatives (17, 18, 40)

Compd	Reagents[a]	Conditions	Cleavage products: 12	4	3	32	Unreacted starting material
			Approximate ratio by vpc				
16	Glacial AcOH	Reflux, 0.5–2.0 hr	1[b]	Trace		3	
	$AcOH–H_2O$	Reflux, 2 hr	1	0.5		1	
	0.13 *M* $HClO_4$ in dioxane–H_2O	Reflux, 1 hr	1	0.5		Trace	0.2
	0.23 *M* $Ba(OH)_2$ in H_2O	100°,[c] 3 hr	1	0.5			
17	Glacial AcOH	Reflux, 2 hr					1
	0.13 *M* $HClO_4$ in dioxane–H_2O	Reflux, 1 hr	1	0.8	0.4	Trace	2[d]
18	Glacial AcOH	Reflux, 6 hr[e]	Trace	Trace	1		0.1
	$AcOH–H_2O$	Reflux, 6 hr[e]	1	3	1		1
	0.13 *M* $HClO_4$ in acetone–H_2O	Reflux, 1 hr[e]	1	0.5			0.3
40	Glacial AcOH	Reflux, 6 hr[e]				Trace	1
	0.13 *M* $HClO_4$ in acetone–H_2O	Reflux, 1 hr[e]					1

[a] Aqueous mixtures were 8.5 AcOH, acetone, or dioxane and 1.5 H_2O (v/v); $HClO_4$ and $Ba(OH)_2$ were 1.5 and 2.5 mol equiv, respectively, per mole of compound. [b] Probably as 1-*O*-acetate (see Experimental Section). [c] Heated on a steam bath. [d] Recovered as **16** (trace of **17**). It is unclear why **3** was formed in this reaction whereas none was found in the reaction of **16** with the same reagent. [e] Product of oxirane opening saponified before analysis by vpc (see Experimental Section for details).

Williams and Bhacca.[22] The H-6 methylene protons of **20** appeared as the AM portion of an AMX system which approached a second-order ABX system. The magnitude of $J_{5,6}$ coupling was used to identify H-6a and H-6e, and it was found that the normal order was reversed, H-6a of **20** appearing downfield from H-6e.

A compound which was formally an anhydro derivative of a 2-hydroxyquinic or 2-hydroxyepiquinic acid was isolated by treating **16** with hot glacial acetic acid. Its nmr spectrum in dimethyl-d_6 sulfoxide at 60 MHz showed a broad resonance at 2.8–3.6 ppm in accord with very rapid exchange of OH and CO_2H protons with those of water of crystallization. A 220-MHz spectrum of the methyl ester tri-*O*-acetate (**36**) is shown in Figure 6 (resonances of $-COCH_3$ protons at δ 1.98–2.06 and $-CO_2CH_3$ protons at δ 3.70 are deleted). Coupling constants are reported in Table I, and dihedral angles calculated from them are compared in Table II with those observed in a Dreiding model of **32**. The spectrum of methyl ester **33** in dimethyl-d_6 sulfoxide clearly showed three secondary hydroxyl protons, indicating the absence of a tertiary hydroxyl on C-1. The above results confirm the 1,4-anhydro structure assigned to **32**.

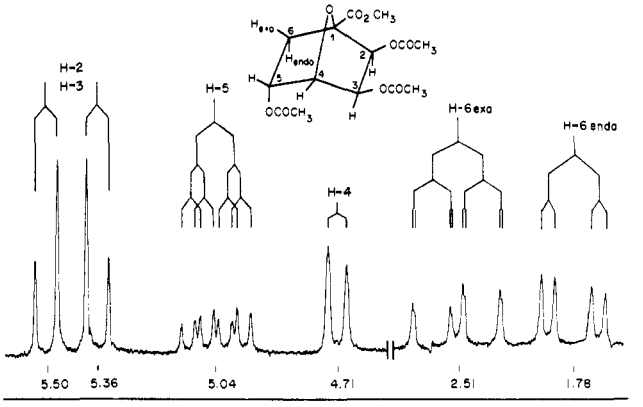


Figure 6. 220-MHz nmr spectrum of methyl 2,3,5-tri-*O*-acetyl-1,4-anhydro-(2*S*)-2-hydroxyquinate (**36**) in $CDCl_3$.

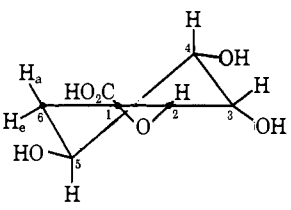
Course of Oxirane Cleavage Reactions. Epoxyshikimic acid (**16**), its tri-*O*-acetate (**18**), and their respective methyl esters **17** and **40** were treated essentially as described under Synthesis, and the course of oxirane cleavage was studied by vpc of trimethylsilyl derivatives. With a few minor exceptions volatile products were derivatives of **3, 32, 4,** and **12** as their lactones (**23** and **19**), and of unreacted oxirane compounds (the trimethylsilyl derivative of the *p*-toluenesulfonate (**21**) was not volatile). Furthermore, **3, 4, 12,** and **32** were not mutually interconvertible under reaction or isolation conditions. The results are summarized in Table III. It was surprising to note that the methyl esters of **16** and **18** did not undergo oxirane cleavage reactions. Thus **17** and **40** were recovered unchanged from refluxing acetic acid, and **40** did not react in dilute perchloric acid at 60° (85% aqueous acetone). In the reaction of **17** with perchloric acid at 100° (85% aqueous dioxane), nearly one-half of the oxirane compound was recovered as free acid, indicating that cleavage occurred only after hydrolysis of **17**. A neighboring carboxyl group was therefore essential for oxirane cleavage. Attractive mechanisms to explain participation by the carboxyl group in oxirane cleavage reactions would appear to be the formation of α- and β-lactone intermediates. However, as discussed below, they do not accommodate all of the results equally well.

Carboxyl group participation would be subject to conformational control, since newly formed groups in epoxide cleavage are in trans-diaxial arrangement.[23] Preferred conformer **16a** would, therefore, be expected to react at C-2, and attack by the carboxyl group or carboxylate ion with inversion would yield the β-lactone **41** *via* a boat-like transition state. Similarly, **16c** would react at C-1 with inversion to give the α-lactone **42**. In glacial acetic acid, **16** was converted predominantly to the 1,4-anhydro compound **32**, which is

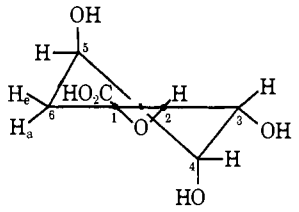
(22) D. H. Williams and N. S. Bhacca, *J. Amer. Chem. Soc.*, **86**, 2742 (1964).

(23) A. Fürst and P. A. Plattner, Abstracts of Papers, 12th International Congress of Pure and Applied Chemistry, New York, N. Y., 1951, p 405.

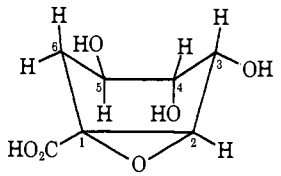
readily accounted for as a product of intramolecular attack, with inversion, at C-1 of the α-lactone (**42**) by the hydroxyl on C-4, thus resulting in the observed net retention of configuration at C-1 of **32**. The nmr



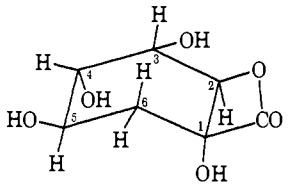
16a



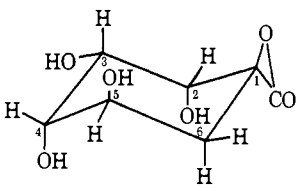
16c



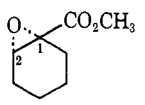
16b



41



42



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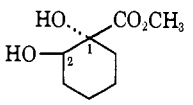

44

spectrum of **16** in ^2H_2O and of **17** in ^2H_2O–acetone–pyridine showed that conformer **a** was preferred in solution. Hence, α-lactone formation must be assumed to prevail over the unfavorable equilibrium between conformers.

A minor product in the reaction of **16** with glacial acetic acid was an acetate ester of the γ-lactone of **12**. Since the main product obtained with *p*-toluenesulfonic acid was the 1-*O*-tosylate of **19**, the acetate lactone is most likely the 1-*O*-acetate. Formation of 1-*O*-acyl derivatives of **12** can be explained by assuming a tertiary carbonium ion intermediate at C-1, shielded by oxirane oxygen, which undergoes nucleophilic attack in a concerted mechanism. The external nucleophile may be hydrogen bonded to the carboxyl group, and is therefore directed to attack opposite to the oxirane ring, with resulting inversion of configuration. Alternatively, formation of 1-*O*-acyl derivatives of **12** can be explained by acyl–oxygen fission[24] of the α-lactone intermediate **42** by acetic or *p*-toluenesulfonic acid, and esterification by the resulting anhydride of the neighboring hydroxyl at C-1.

In 85% aqueous acetic acid the yield of **32** was considerably reduced, and **19** was obtained, presumably by direct hydrolysis of the α-lactone **42**, as well as a smaller fraction of **4** which would be expected from hydrolysis of the β-lactone **41** by carbonyl–oxygen fission. This type of cleavage of **16** with inversion of configuration at C-1 and to a smaller extent at C-2 occurred also with perchloric acid in 85% aqueous dioxane and with aqueous $Ba(OH)_2$ (Table III). Although unreactivity of the methyl ester **17** cannot be shown in base, it is reasonable to assume that oxirane cleavage by aqueous $Ba(OH)_2$ occurred with carboxylate ion participation.

On the other hand, **18** reacted with glacial acetic acid to give, after saponification, almost entirely (2*S*)-2-hydroxyquinic acid (**3**). This unusually stereoselective course of oxirane opening can be explained by formation with inversion of α-lactone (**42**) and/or β-lactone (**41**) intermediates which are attacked with inversion by acetic acid at C-1 and C-2, respectively, to yield a tetra-*O*-acetate of **3**. Overall net retention of configuration would result in either case. Intramolecular attack at C-1 of **18** by the 5-*O*-acetoxy group to yield an acetoxonium ion intermediate which then reacts at C-1 with acetic acid is apparently precluded by the complete lack of reactivity of **40** with this reagent. In 85% aqueous acetic acid only a small fraction of **3** was formed; the major product was **4**, resulting from hydrolysis of the β-lactone, accompanied by a smaller amount of **12** from hydrolysis of the α-lactone intermediate. With perchloric acid in 85% aqueous acetone the pattern of products was similar to that obtained with **16**.

It is noteworthy that the reaction of **18** with glacial acetic acid is much slower than that of **16**, since a small amount of **18** remained after 6 hr at reflux temperature while the reaction of **16** was essentially complete after 0.5 hr. This observation is in accord with the entirely different course of reaction of **16** and **18** in glacial acetic acid. The absence of **3** among the reaction products of **16** with glacial acetic acid suggests that attack on the α-lactone **42** by an external nucleophile at C-1 is restricted owing to steric hindrance at an already crowded quaternary carbon. It is apparently easier for an internal nucleophile to attack at C-1 of **42** to give **32** as the major product. However, with **18** intramolecular attack is impossible, and if the same restriction is valid at C-1, **3** results from the presumably much slower attack of acetic acid at C-2 of **41**.

The proposed mechanisms are necessarily preliminary in nature. The results were obtained mostly in efforts to prepare the desired products, and nothing is known about the kinetics of the reactions, nor about the path of oxygen in oxirane cleavage of either **16** or **18**. There are only a few studies of model cyclohexanecarboxylic acid 1,2-epoxides; the methyl ester **43** was cleaved with perchloric acid in aqueous acetone to give a high yield of the trans-diaxial glycol of the ester **44**.[12] The surprising stabilities of the oxirane ring of **17** and **40** appear therefore to be related to substitution by electronegative groups on the cyclohexane ring.

Experimental Section

General. Melting points were taken on a Fisher-Johns block and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 21 instrument in KBr (unless otherwise stated), and absorptions are given in microns. Nmr spectra were obtained at 60 MHz unless otherwise indicated. Chemical shifts are given in parts per million downfield from an internal TSS or tetramethylsilane standard. All evaporations were carried out at reduced pressure. Elemental analyses were performed by Galbraith

(24) α-Lactone intermediates were invoked in α-halo acid displacements in order to rationalize a net retention of configuration by a double inversion mechanism.[25] Hence, it was postulated that a nucleophilic attack with inversion occurred at the alkyl carbon of the α-lactone. However, there appears to be no *a priori* reason why α-lactones cannot be attacked by nucleophilic reagents at the acyl carbon.

(25) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill, New York, N. Y., 1940, p 177.

Laboratories, Inc., Knoxville, Tenn. Thin layer chromatography (tlc) was done on 20 cm^2 plates of silica gel S-HR (Brinkman Instruments, Inc.). Approximately 0.1 *M* solutions in 50% ethanol were applied to the plates, which were chromatographed for 2–3 hr in *n*-butyl alcohol–water–acetic acid (3:2:1, v/v). They were then dried and sprayed with glycol reagent.[26] Lactones were detected by the hydroxamic acid test.[27] Vapor phase chromatography (vpc) was carried out on trimethylsilyl derivatives[28] prepared from 1–2 mg of pure compounds, material from reaction mixtures, or resolved bands from tlc. A Chromolab instrument was used with Argon ionization detector and a 60 × 1/4 in. column of 1% SE-30 on Chromosorb W. Oxidations with periodate were carried out by standard procedures.[29] Ethanol refers to commercial absolute alcohol. Methyl esters were prepared by treating a methanolic solution of the acid with an excess of diazomethane in ether. γ-Lactone acetates **27**, **25**, **31**, **20**, and **22** were prepared by refluxing **3**, **23**, **10**, **19**, and **21**, respectively, with a large excess of acetic anhydride for 3 hr under moisture exclusion. Acetic anhydride was removed, and the residue crystallized from 95% ethanol.

Shikimic acid was in part a gift from Professor Y. Hirata, Nagoya University, and in part was isolated from the dried fruit of *Illicium religiosum*. The seeds were discarded, and 600 g of the ground carpels were stirred with 2 l. of 95% ethanol for 2 days at room temperature. The suspension was filtered, the brown extract evaporated to dryness, and the residue allowed to crystallize slowly at room temperature for 1 hr. Water was added twice and evaporated to dryness, and the residual solid (60 g) was stirred with 600 ml of water for 1 hr at room temperature. A brown gelatinous material was removed by filtration on a large Büchner funnel, and the filtrate stirred for 1 hr with a little charcoal. The clear solution was evaporated to dryness, and the crude shikimic acid dried for 2 days *in vacuo* (first over NaOH and then over P_2O_5) and crystallized by gradual chilling from 300 ml of hot glacial acetic acid. The crystals were washed with cold acetic acid, cold ethanol, and cold ether: 16 g of a slightly tan product; mp 184°. The ground carpels were reextracted with 1.5 l. of 95% ethanol, and following the above procedure gave 25 g of crude solid from which were obtained 6.7 g of almost white product: mp 184°. A larger scale operation gave higher yields; *e.g.*, 200 g of crude solid gave 127 g of shikimic acid.

1,2-Anhydro-(2*S*)-2-hydroxyquinic (Epoxyshikimic) Acid (16). A. **From 2-Bromoquinolactone[30] (15).** A stirred suspension of 1.27 g (5 mmol) of **15**[14] in 15 ml of H_2O, kept in an ice bath and protected from CO_2 with a soda lime tube, was treated slowly (about 1 hr) with 37.3 ml of 0.132 *M* $Ba(OH)_2$ (5 mmol). The reaction mixture was kept at pH 10.0–10.5 for most of the addition, and finally at pH 11. The almost clear solution was kept at ambient temperature for 2 hr, filtered, and stirred with Dowex 50-H^+ (12 ml wet) to pH 2. After removing and washing the resin the combined filtrates were added to a slight excess of Ag_2O (prepared from 15 ml of 0.4 *M* $AgNO_3$ and 15 ml of 0.4 *M* NaOH), and shaken vigorously for about 20 min. AgBr was removed by filtration on a fritted glass funnel, and the cloudy filtrate was treated with a little charcoal and then with small amounts of Dowex 50-H^+ until a negative test was obtained for Ag^+. The clear solution was concentrated to a thick syrup which crystallized partly on standing for 2 hr at room temperature. Repeated addition and evaporation of ethanol and acetone afforded a crystalline material, mp 145° dec. After drying *in vacuo* overnight the product was dissolved in 30 ml of hot ethanol, and the solution was filtered, chilled, and treated slowly with 30 ml of ligroin (bp 65–75°). An amorphous precipitate was removed and discarded, and ligroin was added with chilling until a faint cloudiness persisted (about 30 ml). After chilling overnight at 2–4°, crystalline material was collected and washed with cold ethanol–ligroin (1:4): yield 0.58 g (61%); mp 145°.

B. **From 1,2-Dibromoshikimic Acid (13).** A stirred solution of 10.0 g (30 mmol) of **13** in 200 ml of H_2O (protected from light) was treated with 4.14 g (15 mmol) of Ag_2CO_3, and stirring was continued for 5 hr at room temperature. The filtrate and washings from removal of AgBr were concentrated to about 90 ml, chilled in an ice bath, and treated with 233 ml of 0.129 *M* $Ba(OH)_2$ (30 mmol) as described above. The pH rose to 9 after addition of about one-half the base (10 min), and 1 hr was required to add the remainder while keeping the solution around pH 11. The yellow solution was kept for 2 hr at room temperature and treated as described above under A. The product was recrystallized from ethanol–ligroin (125:250, ml:ml) and afforded 3.6 g (63%): mp 144–145°; ir 5.83, 7.10, 8.25, 9.65, 10.83, 12.50; $[\alpha]^{27}D$ −92° (*c* 2.1, H_2O); nmr, see Figure 1.

Anal. Calcd for $C_7H_{10}O_6$: C, 44.22; H, 5.30; O, 50.48. Found: C, 44.49; H, 5.35; O, 50.16.

Methyl epoxyshikimate (17) was crystallized from ethyl acetate: mp 108–109°; nmr (^2H_2O, acetone-d_6, pyridine-d_5; 100 MHz) δ 2.6 [d, $J = 0.5(J_{5,6a} + J_{5,6e}) = 5$ Hz, H-6a,6e], 3.64 (s, 3 H), 3.75 (d, J = 3.5 Hz, H-2), 3.9 (d of d, J = 5 and 7.5 Hz, H-4), 4.2 (d of t, J = 5 and 7.3 Hz, H-5), 4.55 (d of d, J = 3.5 and 5 Hz, H-3); $[\alpha]^{27}D$ −100.5° (*c* 1.25, H_2O).

Anal. Calcd for $C_8H_{12}O_6$: C, 47.06; H, 5.92. Found: C, 47.44; H, 5.90.

3,4,5-Tri-*O*-acetylepoxyshikimic Acid (18). A solution of 950 mg (5 mmol) of **16** in 7 ml of dry pyridine was treated at room temperature with 7 ml of acetic anhydride under moisture exclusion, stirred for 20 min, allowed to stand for 20 hr, and concentrated to a small volume. The residue was treated twice with water which was evaporated and dissolved in 20 ml of $CHCl_3$, and the solution was stirred with 5 ml of H_2O for 1 hr to destroy acetic anhydride. The $CHCl_3$ layer was washed with 5 ml of cold water, dried over $MgSO_4$, and concentrated to a syrup which was dried for 2 days *in vacuo* over P_2O_5 and NaOH: yield 1.2–1.5 g (76–94%) of a glass which could not be crystallized; ir ($CHCl_3$) 5.72 (sh, 5.59), 7.04, 7.31 (v strong).

Methyl 3,4,5-Tri-*O*-acetylepoxyshikimic Acid (40). A solution of **17** in dry pyridine was acetylated as described above for **18**. The chloroform solution was washed with water, cold 2 *N* H_2SO_4 to acid pH, 5% $NaHCO_3$ to alkaline pH, and again with water, dried over $MgSO_4$, and concentrated to a syrup which was dried *in vacuo* over P_2O_5 and NaOH. The product could not be crystallized: ir 5.71, 5.74, 6.97, 7.31 (v strong).

(2*S*)-2-Hydroxyepiquino-3-lactone (19). A solution of 950 mg (5 mmol) of epoxyshikimic acid (**16**) in 55 ml of 0.23 *M* $Ba(OH)_2$ (12.5 mmol) was left overnight at room temperature, and heated on a steam bath for 3 hr under N_2. A small amount of tar was removed by filtration, the slightly yellow filtrate was brought to pH 2 by stirring with Dowex 50-H^+ (30 ml wet), and the resin was removed by filtration and washed. The combined filtrate and washings were treated with a small amount of charcoal, the colorless filtrate was concentrated to dryness, and the residue was dried by several additions and evaporations of ethanol. Drying overnight over P_2O_5 *in vacuo* gave 950 mg of a syrup, which by vpc contained the 3-lactone of **12** and the 5-lactone of **4** in a ratio of 2:1. The syrup was induced to crystallize by the addition of a few drops of ethanol and standing at room temperature. The crystalline mass was triturated with 12 ml of cold ethanol, and the crystals were removed by filtration and washed with 2.5 ml of cold ethanol, and then cold ether: yield 210 mg; mp (unsharp) 190° dec. The filtrate was evaporated to dryness and another crop having the same melting point was obtained by the same procedure: total yield 350 mg (37%); ir 5.64 (γ-lactone); nmr (^2H_2O) δ 2.15 (m, H-6a, 6e), 3.72 (m, H-4, H-5), 4.2, 4.7 (2s, H-2, H-3); $[\alpha]^{26}D$ −69° (*c* 1.03, 50% aqueous ethanol).

Anal. Calcd for $C_7H_{10}O_6$: C, 44.22; H, 5.30; O, 50.48. Found: C, 44.44; H, 5.21; O, 50.40.

Mother liquors from isolation of **19** were concentrated to dryness, and the residue was dried *in vacuo* over P_2O_5 to give 510 mg of a syrup which by vpc contained the 3-lactone of **12** and the 5-lactone of **4** in a ratio of approximately 1:1. This material was used as described below for the isolation of **25**.

1,2,4,5-Tetra-*O*-acetyl-(2*S*)-2-hydroxyepiquino-3-lactone (20). A solution of 140 mg of lactone **19** in 3 ml of acetic anhydride was refluxed for 3 hr, and evaporated to dryness. The residue was freed of acetic anhydride by repeated addition and evaporation of 95% ethanol. The crystalline residue was recrystallized from 95% ethanol: yield 180 mg (67%); mp 192–193°; ir 5.52 (γ-lactone), 5.60 (secondary acetate), 5.74 (sh, tertiary acetate), 7.0, 7.30 (v strong); nmr, see Figure 2.

Anal. Calcd for $C_{15}H_{18}O_{10}$: C, 50.28; H, 5.06; O, 44.65. Found: C, 50.18; H, 5.16; O, 44.82.

(2*S*)-2-Hydroxy-1-*O*-*p*-toluenesulfonylepiquino-3-lactone (21). A solution of 190 mg (1 mmol) of **16** and 190 mg (1 mmol) of *p*-toluenesulfonic acid monohydrate in 10 ml of anhydrous dioxane

(26) W. E. Trevelyan, D. P. Proctor, and J. S. Harrison, *Nature (London)*, 166, 444 (1950).

(27) M. Abdel-Akher and F. Smith, *J. Amer. Chem. Soc.*, 73, 5859 (1951).

(28) C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *ibid.*, **85**, 2497 (1963). The reagents were hexamethyldisilazane and trimethylchlorosilane in anhydrous pyridine.

(29) R. D. Guthrie, *Methods Carbohyd. Chem.*, 439 (1962).

(30) Nmr (^2H_2O) δ 2.65 [d, J = 0.5 ($J_{5,6a} + J_{5,6e}$) = 3 Hz, H-6a, 6e], 3.9 (d of d, J = 10 and 4.5 Hz, H-3), 4.82 (d of d, J = 4.5 and 6 Hz, H-4), 4.86 (d, J = 10 Hz, H-2), 4.94 (m, H-5). See Tables I and II for coupling constants and dihedral angles.

was refluxed for 5 hr and evaporated to dryness. Alcohol was added twice to the residue and evaporated to dryness, and the crystals were triturated with about 1 ml of cold ethyl acetate, collected, and washed with cold ethyl acetate and cold ether: yield 156 mg (45%); mp 210–212° dec. An analytical sample was obtained by cooling a hot methanolic solution of the above material to room temperature, and adding ether followed by ligroin to turbidity: mp 235° dec; ir 5.57 (γ-lactone), 7.42, 8.17 (asymmetrical and symmetrical $>SO_2$); nmr (^2H_2O, acetone-d_6) δ 2.34 (m, H-6a, 6e), 2.45 (s, 3 H), 3.81 (m, H-4, H-5), 4.61, 4.68 (2s, H-2, H-3), 7.44, 7.91 (A_2B_2, 4 H, J = 8.5 Hz); $[\alpha]^{28}D$ −29° (*c* 1.0, 95% ethanol).

Anal. Calcd for $C_{14}H_{16}O_8S$: C, 48.90; H, 4.69; O, 37.10; S, 9.30. Found: C, 48.93; H, 4.70; O, 37.08; S, 9.37.

The residue obtained by evaporating the mother liquors from the crude product contained by vpc **19** as well as a smal amount of an unknown material. The trimethylsilyl derivative of **21** was not volatile. A lower yield (28%) of crude **21** was obtained from methyl epoxyshikimate (**17**) treated as described above, except that the reaction mixture was refluxed for 20 hr. Since a saponification step was not required the ester was probably hydrolyzed prior to oxirane cleavage.

2,4,5-Tri-*O*-acetyl-1-*O*-*p*-toluenesulfonyl-(2*S*)-2-hydroxyepiquino-3-lactone (22) showed: mp 169°; ir 5.47 (γ-lactone), 5.71 (secondary acetate), 7.31 (v strong); nmr ($CDCl_3$) δ 2.05 (6s, 9 H,3-$COCH_3$), 2.43 (s, 3 H), 2.6 (m, H-6a, 6e), 4.78 (s, H-3), 5.2 (m, H-4, H-5), 5.7 (s, H-2), 7.3, 7.8 (A_2B_2, 4 H, J = 8 Hz).

Anal. Calcd for $C_{20}H_{22}O_{11}S$: C, 51.1; H, 4.71; S, 6.82. Found: C, 51.4; H, 4.54; S, 6.57.

3,4-*O*-Isopropylidene-(2*R*)-2-hydroxyquino-5-lactone (24). The syrup (1.12 g) from two identical preparations of **19** (see above) was treated at room temperature with 110 ml of 1% HCl in acetone, stirred until solution had taken place (30 min), and allowed to stand for 24 hr. A 0.25-ml aliquot was evaporated to dryness, and the residue redissolved in acetone and evaporated again to dryness. Vpc of the trimethylsilyl derivatives showed a small amount of unreacted lactones, and approximately half of the starting material as the 3,4-*O*-isopropylidene-5-lactone of **4** with a retention time of 5.0 min. A shoulder with a retention time of 5.5 min was also present, and was identified as an isopropylidene derivative of **19**.[31]

The acetone reaction mixture was brought to neutrality by stirring with a slight excess of Ag_2CO_3 for 0.5 hr, and silver salts were removed by filtration. The slightly cloudy filtrate was clarified with a little charcoal, and evaporated to dryness: yield 1.08 g of a crystalline mixture. Extraction of the solid at room temperature with ethyl acetate (10-, 10-, 5-, and 3-ml portions) and evaporation of the solvent gave 640 mg of crude acetonide. Recrystallization from 20 ml of ethyl acetate gave a first crop of 110 mg: mp 205° (a second crop, 96 mg, and a third crop, 55 mg, were slightly contaminated with free lactones); ir 5.58 (γ-lactone), 7.21, 7.29 (*gem*-dimethyl).

Anal. Calcd for $C_{10}H_{14}O_6$: C, 52.17; H, 6.13; O, 41.70. Found C, 52.17; H, 6.22; O, 41.82.

1,2,3,4-Tetra-*O*-acetyl-(2*R*)-2-hydroxyquino-5-lactone (25). A solution of 46 mg (0.2 mmol) of **24** in 6 ml of 80% acetic acid was heated 2.5 hr on the steam bath and evaporated to dryness, and the residue was freed of acetic acid and water by repeated addition and evaporation of H_2O, 95% ethanol, and ethanol. The syrup was dried *in vacuo* over P_2O_5, and acetylated as described previously for the preparation of **20** to give 67 mg (93%) of a crystalline product, which was recrystallized from 2.5 ml of 95% ethanol: yield 39 mg; mp 196–197°; ir 5.52 (γ-lactone), 5.70 (secondary acetate), 5.72 (tertiary acetate); nmr, see Figure 3.

Anal. Calcd for $C_{15}H_{18}O_{10}$: C, 50.28; H, 5.06; O, 44.65. Found: C, 50.46; H, 4.82; O, 45.01.

(2*S*)-2-Hydroxyquinic Acid (3). A solution of 1.3 g (4.2 mmol) of **18** in 40 ml of glacial acetic acid was refluxed for 6 hr under N_2 and exclusion of moisture, and evaporated almost to dryness. Water, 95% ethanol, and ethanol were added several times and removed by evaporation, and the syrup was dried *in vacuo* over P_2O_5. A solution of the product in 126 ml of 0.2 *N* KOH in 50% aqueous methanol was stirred overnight, and treated with Dowex 50-H^+ (30 ml of wet resin) to pH 2. Filtrate and washings were evaporated to dryness, and the mostly crystalline residue was dried *in vacuo* over P_2O_5: yield 0.74 g (85%). Recrystallization from about 10 ml of 75% ethanol gave 238 mg: mp 221°. A second crop (96 mg) was obtained from the mother liquors: mp 219–220° (total yield 38%); ir 5.78 (CO_2H), 6.85; $[\alpha]^{27}D$ −16° (*c* 1.0, 50% ethanol).

Anal. Calcd for $C_7H_{12}O_7$: C, 40.39; H, 5.81; O, 53.80. Found: C, 40.50; H, 5.80; O, 53.78.

A solution of **40** in glacial acetic acid was treated as described above for **18**. Vpc showed the presence of only epoxyshikimic acid (**16**).

Methyl (2*S*)-2-hydroxyquinate (26) was recrystallized from ethanol: mp 170–171°.

Anal. Calcd for $C_8H_{14}O_7$: C, 43.25; H, 6.35; O, 50.40. Found: C, 43.45; H, 6.45; O, 50.38.

1,2,3,4-Tetra-*O*-acetyl-(2*S*)-2-hydroxyquino-5-lactone (27). A syrup was obtained: ir 5.53 (γ-lactone), 5.69 (secondary acetate), 5.72 (tertiary acetate); nmr, see Figure 4.

Methyl 1,2:3,4-Di-*O*-isopropylidene-(2*S*)-2-hydroxyquinate (28). A suspension of 111 mg (0.5 mmol) of **26** in 10 ml of 1% HCl in acetone was stirred for 4 days at room temperature. The solution was stirred with a small excess of Ag_2CO_3, and the precipitate was removed by filtration and washed. The filtrate and washings were stirred with a small amount of charcoal, and the clear solution was evaporated to dryness. The residual syrup was dried *in vacuo* over P_2O_5: yield 110 mg (70%); ir 5.70, 5.75 (d for $-CO_2CH_3$), 6.84, 6.95, 7.24, 7.27, and 7.31 (diisopropylidene); nmr ($CDCl_3$) δ 1.34, 1.38, 1.52 (3 s, 12 H, two isopropylidene groups), 1.85 (m, H-6a, 6e), 3.8 (s, 3 H), 4.34 (m, H-2, H-3, H-4, H-5).

Reaction of 3,4,5-Tri-*O*-acetylepoxyshikimic Acid (18) and of Its Methyl Ester (40) with Perchloric Acid. A solution of 5 mmol of **18** and 7.5 ml of 1 *N* $HClO_4$ in 50 ml of acetone was refluxed for 1 hr. Perchlorate ion was removed as the potassium salt by adding an equimolar amount of $KHCO_3$ in a small volume of water, and the resulting product was hydrolyzed with KOH in 50% aqueous methanol and freed of K^+ with Dowex 50-H^+. A nearly quantitative yield of a syrup was obtained assuming oxirane ring cleavage, ester hydrolysis, and lactonization. Vpc of the trimethylsilyl derivatives showed the lactones of **12** and **4** in a ratio of approximately 2:1, and a small amount of starting material. The methyl ester **40** was treated with perchloric acid in acetone and with KOH as described above for the acid **18**. Vpc showed **16** as the only product. After 8 hr at reflux temperature and hydrolysis with KOH 50% of starting material was recovered as epoxyshikimic acid (**16**). Vpc of the mother liquors showed the lactones of **4** and **12** in a ratio of 1:1.

(2*R*)-2-Hydroxyepiquinic Acid (10). A. **From 9.** The starting material was prepared by the procedure of Fischer and Dangschat;[5] mp 134–135°; $[\alpha]^{25}D$ −13.5° (*c* 1.12, MeOH); nmr ($CDCl_3$; 100 MHz) δ 1.30, 1.50 (2 s, 6 H, isopropylidene), 1.78 (d of d, J = 7.5 and 14 Hz, H-6), 2.05 (s, 3 H), 2.5 (d of d, J = 6 and 14 Hz, H-6), 3.78 (s, 3 H), 3.92 (d, J = 7.5 Hz, H-2), 4.32 (m, H-3, H-4), 5.07 (m, J = 6, 6.5, and 7.5 Hz, H-5). A solution of 913 mg (3 mmol) of **9** in 40 ml of 80% acetic acid was kept overnight at room temperature, and evaporated to dryness. The syrupy residue was treated several times with ethanol which was evaporated, and left overnight at room temperature in 45 ml of 0.2 *N* KOH in 80% MeOH. K^+ ions were removed with Dowex 50-H^+, and the filtrate and washings were evaporated to dryness. Addition of a few drops of ligroin promoted crystallization of the residue, and recrystallization from 35 ml of ethanol by addition of ligroin (about 30 ml) to a faint turbidity afforded 343 mg (55%); mp 187–188°. A sample for analysis was prepared by recrystallizing from ethanol–ligroin and drying carefully *in vacuo* over P_2O_5 at 100° for 3 hr: mp 188°; ir 5.75; $[\alpha]^{26}D$ +42° (*c* 1.25, H_2O).

Anal. Calcd for $C_7H_{12}O_7$: C, 40.39; H, 5.81; O, 53.80. Found: C, 40.32; H, 5.59; O, 52.54.

B. **From Methyl Shikimate (7).** A cold solution of 376 mg (2 mmol) of methyl shikimate[5] and 0.38 ml (4.7 mmol) of dry pyridine in 80 ml of ethyl acetate was treated with 508 mg (2 mmol) of OsO_4 in 1.5 ml of ethyl acetate, and left for 12 days at room temperature in the dark.[6] The reaction mixture was chilled in an ice bath, and the osmate ester was removed by filtration and washed with anhydrous cold ether. The powdery product was dissolved *immediately* in about 50 ml of ethanol–CH_2Cl_2 (1:1) and treated for a few minutes with a stream of H_2S. The osmium sulfide was removed by centrifugation, and the yellow supernatant solution was treated with a little charcoal, filtered, and evaporated to dryness. The methyl ester was hydrolyzed with 10.5 ml of 0.2 *N* NaOH in 80% aqueous MeOH as described above under A, and gave 290 mg (70%) of crystalline product, which was recrystallized from ethanol–ligroin: yield 80 mg (27%); mp 188°. This material was identical with that obtained in A.

(31) An authentic sample of this derivative (mp 175°) was prepared directly from **19**, and showed γ-lactone and *gem*-dimethyl absorptions in the ir. Examination of a Dreiding model of **19** revealed that the trans equatorial hydroxyl groups at C-4 and C-5 are close enough to form an acetonide, owing to the partial flattening of the chair conformation of the cyclohexane ring by the γ-lactone.

Methyl (2*R*)-2-hydroxyepiquinate (**29**) was recrystallized from ethanol: mp 135–136°.

Anal. Calcd for $C_8H_{14}O_7$: C, 43.25; H, 6.35; O, 50.40. Found: C, 43.23; H, 6.34; O, 50.14.

Methyl 1,2:3,4-Di-*O*-isopropylidene-(2*R*)-2-hydroxyepiquinate (**30**). A solution of 50 mg of ester **29** in 5 ml of a 1% solution of HCl in acetone was stirred for 4 days at room temperature, and treated as described for preparation of **28**: yield 63 mg (71%) of a syrup which could not be crystallized; ir 5.73 (ester), 7.24, 7.27, 7.30 (di-*O*-isopropylidene); nmr ($CDCl_3$) δ 1.38, 1.45, 1.52 (3 s, 12 H, two isopropylidene groups), 2.17 (m, H-6a, 6e), 3.81 (s, 3 H), 4.28 (m, 2 H), 4.6 (d of d, J = 2 and 7 Hz, 1 H), 4.82 (d, J = 2 Hz, 1 H).

1,2,4,5-Tetra-*O*-acetyl-(2*R*)-2-hydroxyepiquino-3-lactone (**31**). A solution of the acid **10** in a tenfold excess of acetic anhydride was refluxed for 3 hr, and treated as previously described. The crystalline product was recrystallized from 95% ethanol: mp 142–143°; ir 5.57 (γ-lactone), 5.68 (secondary acetate), 5.72 (tertiary acetate); nmr, see Figure 5.

Anal. Calcd for $C_{15}H_{18}O_{10}$: C, 50.28; H, 5.06; O, 44.65. Found: C, 50.29; H, 5.16; O, 44.77.

1,4-Anhydro-(2*S*)-2-hydroxyquinic Acid (**32**). A solution of 1.9 g (10 mmol) of 1,2-epoxyshikimic acid (**16**) in 50 ml of glacial acetic acid was refluxed for 2 hr under N_2 and exclusion of moisture, and evaporated to dryness. The residue was treated sequentially with water and ethanol which were evaporated, and the partially crystalline product was dried *in vacuo* over P_2O_5. Trituration with 95% ethanol and with ether gave a small amount of a product, mp 113°, which was identified by nmr as **32**. Analysis of the reaction mixture by vpc showed **32** as the main component accompanied closely by another compound in a ratio of 3:1, respectively, and ir showed **32** plus a monoacetate of a γ-lactone. An ethereal solution of the reaction mixture was treated with diazomethane, the solvent was removed, and the dried residue was treated with acetic anhydride and pyridine as described for preparation of **40**. Nmr of the product showed **32** as the methyl ester tri-*O*-acetate (**36**), and **12** as the lactone tetra-*O*-acetate (**20**). Since **32** was obtained in poor yield by crystallization of the crude residue from 95% ethanol, the residue was treated with 175 ml of 0.2 *N* KOH in 80% methanol as described previously, and the mostly crystalline product was triturated with 10 ml of cold 95% ethanol: yield 830 mg (40%). Recrystallization from 20 ml of 95% ethanol gave 690 mg (33%): mp 114–116°. An analytical sample was prepared by another recrystallization from 95% ethanol: mp 115°; ir 5.60, 6.88, 7.22, 7.80, 8.05, 8.93, 9.05, and 9.57; nmr (^2H_2O) δ 1.5 (d of d, J = 3.5 and 14 Hz, H-6 endo), 2.35 (m, H-6 exo), 4.32 (m), 4.6 (d) (H-2, H-3, H-4, H-5); $[\alpha]^{26}D$ +19.40° (*c* 1.095, H_2O).

Anal. Calcd for $C_7H_{10}O_6 \cdot H_2O$: C, 40.39; H, 5.81; O, 53.80. Found: C, 40.33; H, 5.84; O, 53.73.

Examination by vpc of the reaction of **16** with glacial acetic acid for 0.5, 1.0, 2.0, 4.0, and 6.0 hr showed formation of the same products (**32** and an *O*-acetate of **20**) in a ratio of 3:1.

A solution of 50 mg (0.25 mmol) of the methyl ester **17** in 2 ml of glacial acetic acid was refluxed under N_2 and moisture exclusion as described above for preparation of **32** from **16**. Aliquots were withdrawn after 2.5, 5, and 7.5 hr, and evaporated to dryness. Vpc showed that the starting material remained unchanged after 2.5 hr. The 1,4-anhydro compound **32** was not formed in any of the samples, and traces of two unknown products which were not studied further were present in the 5- and 7.5-hr samples.

A solution of 21 mg (0.1 mmol) of **3** was refluxed in 2 ml of glacial acetic as described above. Vpc showed a compound which was not **32**, and treatment with base regenerated **3**, indicating that under conditions of formation of **32**, **3** was lactonized, but was not converted to **32**.

Methyl 1,4-anhydro-(2*S*)-2-hydroxyquinate (**33**) was recrystallized twice from ethanol: mp 162–163°; nmr (^2H_2O) δ 1.5 (d of d, J = 3.5 and 14 Hz, H-6 endo), 2.3 (m, H-6 exo), 3.85 (s, 3 H), 4.3 (m), 4.65 (d) (H-2, H-3, H-4, H-5); $[\alpha]^{27}D$ +25° (*c* 1.3, H_2O).

Anal. Calcd for $C_8H_{12}O_6$: C, 47.06; H, 5.92; O, 47.02. Found: C, 47.09; H, 5.81; O, 47.00.

1,4-Anhydro-2,3-*O*-isopropylidene-(2*S*)-2-hydroxyquinic Acid (**34**). A suspension of 200 mg of **32** in 20 ml of 1% HCl in acetone was stirred for 2 days at room temperature. The clear solution was neutralized with $PbCO_3$, and the filtrate was stirred with a little charcoal, filtered, and evaporated to dryness. The crystalline residue was dried *in vacuo* over P_2O_5: yield 215 mg; mp 198–200°. For analysis a sample was recrystallized by dissolving in hot ethyl acetate, chilling, and adding ligroin to turbidity: mp 203°; ir 5.80, 7.23, 7.30 (*gem*-dimethyl).

Anal. Calcd for $C_{10}H_{14}O_6$: C, 52.17; H, 6.13; O, 41.70. Found: C, 52.16; H, 5.99; O, 41.87.

Methyl 1,4-Anhydro-2,3-*O*-isopropylidene-(2*S*)-2-hydroxyquinate (**35**). A suspension of 204 mg (1 mmol) of the methyl ester **33** in 20 ml of 1% HCl in acetone was stirred for 10 min until solution had taken place, and left for 2 days at room temperature. The solution was neutralized with Ag_2CO_3 and the product isolated as described for the preparation of **28**: yield 235 mg (96%); mp 137°. An analytical sample was prepared by recrystallization from warm ethyl acetate by addition of ligroin to turbidity: mp 137°; ir 5.67, 5.72 (d) ($COOCH_3$), 7.48, and 7.53 (*gem*-dimethyl).

Anal. Calcd for $C_{11}H_{16}O_6$: C, 54.09; H, 6.60; O, 39.30. Found: C, 54.20; H, 6.62; O, 39.55.

The identical compound was prepared by treating **34** with diazomethane.

Methyl 2,3,5-Tri-*O*-acetyl-1,4-anhydro-(2*S*)-2-hydroxyquinate (**36**). A solution of 204 mg (1 mmol) of the methyl ester **33** in 2 ml of dry pyridine was treated under moisture exclusion with 2 ml of acetic anhydride, stirred for 20 min, allowed to stand for 20 hr, and concentrated to dryness. The residue was dissolved in $CHCl_3$ and treated as described previously for preparing **40**. The residue from the chloroform solution was treated with a few drops of 95% ethanol to induce crystallization, evaporated to dryness, and dried *in vacuo* over P_2O_5 and NaOH: yield 277 mg (84%); mp 121–122°. Recrystallization from 5.5 ml of 95% ethanol afforded 182 mg: mp 121–122°; ir ($CHCl_3$) 5.71 (secondary acetate), 5.73 (CO_2CH_3), 6.97, 7.31 (v strong); nmr, see Figure 6.

Anal. Calcd for $C_{14}H_{18}O_9$: C, 50.91; H, 5.49; O, 43.60. Found: C, 51.24; H, 5.28; O, 43.51.

Methyl (2*R*)-2-Bromoquinate (**37**). A stirred solution of 13.4 g (40 mmol) of **13** was treated with 5.5 g (20 mmol) of Ag_2CO_3 as described previously for preparing **16**. The filtrate from removal of AgBr was stirred briefly with charcoal which was removed, and the solution was concentrated *immediately*. The residue was dissolved twice in methanol and the solution evaporated nearly to dryness, and then esterified in 100 ml of methanol with an ether solution of diazomethane. (Operations after removal of AgBr must be carried out rapidly in order to avoid lactonization.) Solvents were removed by evaporation, and the residue was dried overnight over P_2O_5 *in vacuo* and dissolved in 40 ml of hot ethyl acetate. The ester was crystallized by cooling the solution slowly to room temperature (2 hr), and then in an ice bath. The product was collected and washed with cold ethyl acetate and cold ether: yield 5.5 g; mp 112–114°. The filtrate was evaporated to dryness, and the residue crystallized from 20 ml of hot ethyl acetate to give a second crop with the same melting point: total yield 6.4 g (56%). An analytical sample was obtained by two further recrystallizations from ethyl acetate: mp 113–114°; ir 5.75 (CO_2CH_3); nmr (^2H_2O) δ 2.3 (m, H-6a, 6e), 3.82 (s, 3 H), 4.06 (m), 4.4 (s) (H-2, H-3, H-4, H-5); $[\alpha]^{24}D$ −28.8° (*c* 2.06, H_2O).

Anal. Calcd for $C_8H_{13}O_6Br$: C, 33.70; H, 4.59; Br, 28.04. Found: C, 33.85; H, 4.78; Br, 27.93.

Methyl 1,3,4,5-Tetra-*O*-acetyl-(2*R*)-2-bromoquinate (**38**). A stirred solution of 3.0 g (10.5 mmol) of **37** in 22 ml of anhydrous pyridine was treated under moisture exclusion with 23.8 g (233 mmol) of acetic anhydride. The mixture was left at room temperature for 2 days and evaporated to dryness, and the residue was treated as described previously for preparing **40**. The product was dissolved in 10 ml of hot methanol, and crystallized by chilling the solution and adding water (about 1 ml) to turbidity. The crystals were collected, washed with cold 50% methanol, and dried *in vacuo* over P_2O_5: yield 2.93 g; mp 74–75°. A second crop (0.75 g; mp 74°) was obtained from the filtrate: total yield 3.7 g (78%). An analytical sample was prepared by recrystallization from hot methanol by addition of water: mp 74–75°; ir 5.68 (secondary acetate), 5.72 (tertiary acetate), 6.90, 6.96, 7.31 (v strong); $[\alpha]^{24}D$ −59.7° (*c* 2.03, ethanol).

Anal. Calcd for $C_{16}H_{21}O_{10}Br$: C, 42.40; H, 4.67; Br, 17.63. Found: C, 42.6; H, 4.84; Br, 17.6.

Attempts to Prepare Derivatives of 2-Hydroxyquinic Acid by Displacing Br in 37 and 38. Silver and sodium dibenzyl phosphate were refluxed with **38** in dimethylformamide.[32] Extensive aromatization took place. Silver tetrafluoroborate was refluxed with **38** for 4 hr in nitromethane in the hope of obtaining a 1,2-dioxolenium salt which would be hydrolyzed with $NaHCO_3$ to a *cis*-hy-

(32) J. Baddiley, V. M. Clark, J. G. Michalski, and A. R. Todd, *J. Chem. Soc.*, 815 (1949).

droxy ester.[33] Although 70% of the theoretical AgBr was recovered, elimination and aromatization had taken place. Displacement reactions with **37** appeared even less hopeful owing to the possibility of 1,2-epoxide formation. In fact, the methyl ester **17** was first prepared by refluxing silver dibenzyl phosphate with **37** in dry acetonitrile.[32] Extensive aromatization occurred in the reaction of dibromoshikimic acid **13** with silver acetate in moist acetic acid,[9] unlike the formation of *cis*-acetoxycyclohexanol from *trans*-dibromocyclohexane under these conditions,

(33) C. B. Anderson, E. C. Friedrich, and S. Winstein, *Tetrahedron Lett.*, 2037 (1963).

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Kinetics of Carbonyl Addition Reactions. I. A Temperature-Jump Study of Carbinolamine Formation between Piperazine and Pyridine-4-aldehyde

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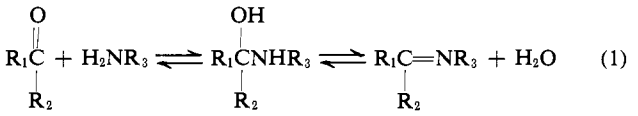
Abstract: Equilibrium and rate constants have been determined for the reactions of piperazine and piperazine monocation with pyridine-4-aldehyde to give the corresponding carbinolamines. The uncatalyzed addition rates for piperazine ($pK = 9.97$), $k_1 = 2\text{–}3 \times 10^5\ M^{-1}\ sec^{-1}$, and for piperazine monocation ($pK = 5.80$), $k_2 = 65\ M^{-1}\ sec^{-1}$, reflect the sensitivity of the rates to the basicity of the attacking amine. The equilibrium constants for carbinolamine formation are much less sensitive to amine basicity. The reaction of piperazine has been shown to be subject to general base catalysis and the reaction of piperazine monocation has been shown to be subject to general acid catalysis. The results are discussed in terms of the detailed mechanism of carbonyl addition reactions.

The reaction of a primary amine with a carbonyl compound to give a Schiff base (eq 1) is now gen-



$$R_1\overset{\overset{O}{\|}}{C}R_2 + H_2NR_3 \rightleftharpoons R_1\overset{\overset{OH}{|}}{C}NHR_3R_2 \rightleftharpoons R_1C{=}NR_3R_2 + H_2O \qquad (1)$$

erally accepted as proceeding through a tetrahedral intermediate.[1] Kinetic evidence for the existence of a carbinolamine intermediate was obtained by French and Bruice[2] in the reaction of pyridine-4-aldehyde with various amino acids. However, they could only infer its presence from evidence of a rapid preequilibrium prior to a rate determining dehydration step to give the Schiff base. Jencks[3] was able to measure the rate of carbinolamine formation in the acid pH range in the reaction of acetone with hydroxylamine. A change in rate limiting step from carbinolamine formation to dehydration was proposed to account for the pH–rate profile. More recently Sander and Jencks[4] have spectrophotometrically confirmed the existence of carbinolamine intermediates using the stopped-flow technique and have measured equilibrium constants for their formation for a series of primary and secondary amines with pyridine-4-aldehyde, formaldehyde, and *p*-chlorobenzaldehyde. However, the rates of carbinolamine formation were too fast to be measured even with the stopped-flow technique. This was also the situation in the recent study by Hine and Via[5] on the reaction of isobutyraldehyde with a series of primary amines.

The work presented in this paper is the first of a series of carbonyl addition reactions involving amines which we have studied by the temperature-jump technique in order to obtain information on those factors which affect the kinetics of these fast addition reactions. Our kinetic program is complementary to that of Jencks on relative stabilities and combined with the kinetic studies of Schuster, *et al.*,[6] on thiol addition reactions will provide a better understanding of the detailed mechanism of these biologically important reactions. The role of acid–base catalysis in these reactions is of particular interest since it may indicate the type of catalysis and the nature of the groups involved when these reactions occur enzymatically.

In this paper details are presented of the kinetics of the reaction of pyridine-4-aldehyde with piperazine in aqueous perchlorate medium. Pyridine-4-aldehyde was chosen as the carbonyl compound for the following reasons: (1) equilibrium constants for carbinolamine formation had been measured for a selection of amines by Sander and Jencks;[4] (2) a strong absorption band in the uv providing a convenient way for spectrophotometric observation of the reaction; (3) related to pyridoxal phosphate (vitamin B_6), an important cofactor in a number of enzyme systems whose mode of action in many instances is thought to involve carbinolamine and Schiff base formation.

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