Acknowledgment.—The competent technical assistance of Mrs. Barbara Tucker is gratefully acknowledged. The author wishes to thank Dr. Robert R. Burtner for the initial suggestion to explore fermentation of the pregnajervanes and for his valued counsel during the course of this work. The preparation of large quantities of intermediates was capably carried out by Mr. R. Salzmann.

The Synthesis of Model Compounds for Maleylacetoacetic Acid. Maleylacetone[1]

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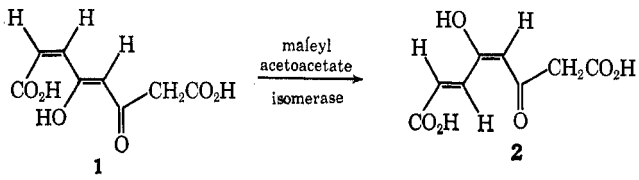
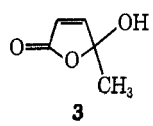
Configurationally labile maleylacetone has been prepared by the careful hydrolysis of 4-acetonylidenebut-2-ene-4-olide (*Z*) and shown to exist as a mixture of closed "pseudo" acid **4a** and open enol acid **4b** in chloroform and benzene and as "pseudo" acid in water. Ultraviolet and nmr spectra of the acids and anions are reported along with dissociation constants of the acid.

In the course of our study of *cis* to *trans* isomerizations we became interested in the mechanism of the enzymatic conversion of maleylacetoacetic acid (**1**) to fumarylacetoacetic acid (**2**). This *cis* to *trans* isomerization occurs in nature in the metabolism of aromatic amino acids.[2]



1 **2**

The study of this mechanism involves the synthesis and investigation of model compounds of **1** having similar but simpler structures. The first and simplest model compound prepared and its mechanism of isomerization studied was *cis*-β-acetylacrylic acid (**3**) which exists as the cyclic "pseudo" acid but as an

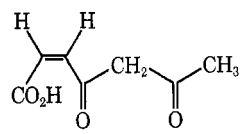


3

acyclic carboxylate anion.[3] The geometrical isomerization is catalyzed by thiocyanate ion which we consider to be a model for glutathione, the coenzyme needed for the enzyme-catalyzed isomerization of maleylacetoacetate. The role of the enzyme in this reaction is more obscure. The enzyme, we have suggested,[4] might catalyze the formation of a Schiff base between maleylacetoacetate and itself. The Schiff base, being more basic, would have a larger fraction protonated than the substrate and this in turn would enhance nucleophilic attack by glutathione thereby to catalyze isomerization.

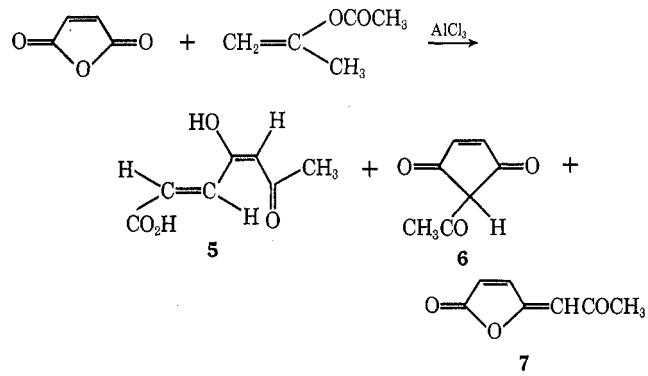
The anion of **3** was examined as to its ability to form Schiff bases with amines.[4] The predominant reaction, unfortunately, appears to be conjugate addition. Those amines possessing an α-nucleophilic atom such as hydroxylamine or semicarbazide, however, do form Schiff bases and the reactivity of the α,β-unsaturated semicarbazone towards thiocyanate was studied. Conjugate addition of amines to the γ,δ double bond in **1** might be less favorable because of the greater delocalization of positive charge and so it was of interest to see the effect of the introduction of an additional keto group, β to the original α′,β′-unsaturated carbonyl group of our first model compound. Moreover, such a molecule would be closer in structure to the natural substrate (**1**) and would be a more accurate model for studying other aspects of the chemistry of **1**.

A compound having the β-diketone moiety α to the *cis* double bond yet being simpler than maleylacetoacetic acid is maleylacetone (**4**). Maleylacetone has



4

previously been reported to have been isolated in 87% purity as a product of the enzymatic oxidation of homogentisic acid.[5] In addition, **4** has been suggested by Nilsson as a probable intermediate in the formation of fumarylacetone (**5**), 2-acetylcyclopentene-1,3-dione (**6**), and 4-acetonylidenebut-2-ene-4-olide (**7**), from the



5 **6** **7**

(1) Research carried out at Brookhaven National Laboratory under contract with the U. S. Atomic Energy Commission.

(2) W. E. Knox in "The Enzymes," Vol. 2, P. D. Boyer, H. Lardy, and K. Myrbäck, Ed., Academic Press, New York, N. Y., 1960, pp 282–289.

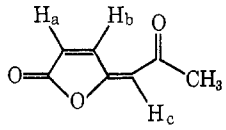
(3) (a) S. Seltzer and K. D. Stevens, *J. Org. Chem.*, **33**, 2708 (1968); (b) K. D. Stevens and S. Seltzer, *ibid.*, **33**, 3922 (1968).

(4) C. Santiago and S. Seltzer, in preparation.

(5) D. I. Crandall, *et al.*, *J. Biol. Chem.*, **235**, 3011 (1960), reported that in the isolation of maleylacetoacetic acid from the enzymatic oxidation of homogentisic acid nonenzymatic decarboxylation occurred to give maleylacetone.

aluminum chloride catalyzed condensation of maleic anhydride and isopropenyl acetate.[6]

We reinvestigated the condensation of isopropenyl acetate with maleic anhydride as a possible synthetic route to maleylacetone. By carrying out the condensation in methylene chloride at room temperature we were able to isolate a butenolide (**8**) isomeric with Nilsson's 4-acetonylidenebut-2-ene-4-olide. The two isomeric butenolides, **7** and **8**, differ in stereochemistry about the *exo* double bond. On examination of their nmr spectra it was possible to determine the structure



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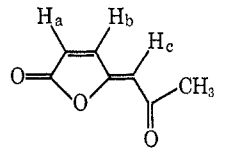
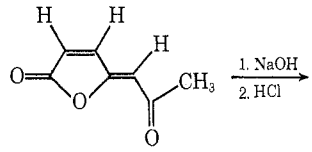


8

of each isomer. The coupling constant of the *exo* vinyl hydrogen (H_c) and H_a was 1.8 Hz in **7** and 0.8 Hz in **8**. Since larger couplings have been observed for protons with *trans* relationships over many bonds than those with *cis* relationships,[7] the structural assignments of 4-acetonylidenebut-2-ene-4-olide (*Z*), **8**, and 4-acetonylidenebut-2-ene-4-olide (*E*), **7**, were made.[8] When **8** is heated in chloroform for 4 hr, 27% of it is converted to **7**. We were not able to isolate **7** following Nilsson's reaction conditions and work-up.[9]

Butenolide **8**, when treated with 1 *N* sodium hydroxide dissolved within a few minutes at room temperature to give an orange solution which upon acidification in the cold followed by rapid extraction with methylene chloride gave maleylacetone as a mixture of the closed "pseudo" acid (**4a**) and open enol acid (**4b**). The aqueous phase of the reaction mixture deposits fumarylacetone (**5**) after standing for several hours at room temperature. The ratio of **4a** to **4b** varies from 3:1 to 15:1 in different reaction mixtures, the former ratio being observed more frequently. We have not determined the cause of the variability of the relative



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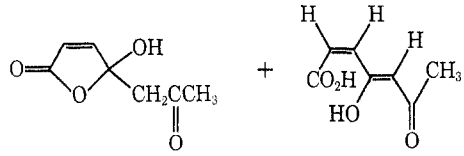
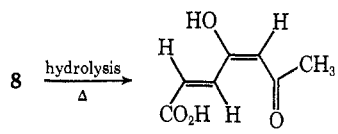


4a **4b**

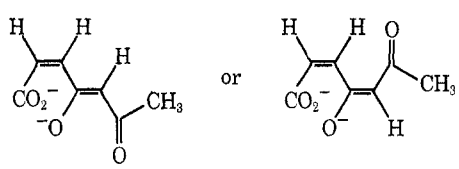
amounts of closed and open acids but suggest that it may be due to variations in both the pH after acidification in the hydrolysis step and the time lapse between acidification and extraction. Owing to the marked tendency for *cis* to *trans* isomerization to occur in maleylacetone, it was not possible to separate the two isomers. Maleylacetone can be stored at −6° for several weeks without appreciable isomerization in fumarylacetone. However, in aqueous solution or to chloroform, isomerization is rapid. For example, when **8** is hydrolyzed and the reaction mixture heated at 100° for a few minutes, fumarylacetone crystallizes from the solution.



6

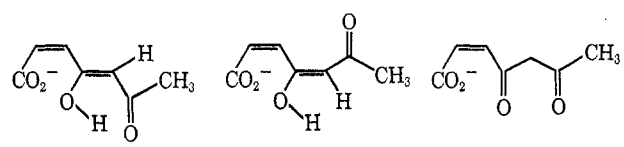
The ratio of **4a** and **4b** was determined from the nmr spectra. The ratio of the two isomers does not vary in chloroform-*d* or benzene-d_6. In water or deuterium oxide, however, maleylacetone exists exclusively as the "pseudo" acid **4a**.

The nmr spectra of the mono and dianions were taken. The dianion was prepared by dissolving the butenolide, **8**, in excess 1 *N* sodium hydroxide. The nmr spectrum showed two singlets in the vinyl region with relative areas of 2:1 Thus the *cis* vinyl protons have the same chemical shift and the presence of a third vinyl proton supports the enolate structure **9**.



9

Acidification of the basic solution gives a solution which has an nmr spectrum identical with that of maleylacetone in water. The monoanion was prepared by adding sodium bicarbonate to a solution of maleylacetone in deuterium oxide (pD of solution 8.34). The spectrum showed two different quartets (J = 12.5 Hz) and two different methyl singlets of equal intensity. The uv spectrum at pH 8.34 exhibits a strong maximum at 312 nm indicating an extended conjugated system as in the enol forms **10a** and **10b**. Since the nmr



10a **10b** **10c**

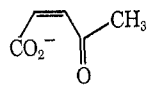
spectra were taken in D_2O, the observation of an additional vinyl proton at C-5 was precluded by its rapid exchange with the solvent. Along with the uv maximum at 312 nm there is also a double humped maximum at 212 and 243 nm which is reminiscent of the uv

(6) M. Nilsson, *Acta Chem. Scand.*, **18**, 441 (1964).

(7) T. Schaefer, *J. Chem. Phys.*, **36**, 2235 (1962); R. T. Hobgood, Jr., and J. H. Goldstein, *J. Mol. Spectrosc.*, **12**, 76 (1964).

(8) Recently, J. E. Blackwood and coworkers [*J. Amer. Chem. Soc.*, **90**, 509 (1968)] introduced this nomenclature for the unambiguous specification of stereoisomerism about a double bond.

(9) Nilsson isolated butenolide **7** as a neutral compound in the reaction of maleic anhydride with isopropenyl acetate. The work-up involved separating **6** and **7** by extracting **6** from the organic phase with 2 *M* sodium hydroxide. Since **7**, like **8**, reacts with base to give maleylacetone, it would appear that it would only be possible to isolate **7** as a neutral compound if the extraction with base were carried out so rapidly that the hydrolysis of **7** is incomplete. Possibly this is the reason that we were not able to isolate **7** under Nilsson's reaction conditions.



11

spectrum of *cis*-β-acetylacrylate ion (**11**). Its spectrum has the same appearance with equal maxima at about 210 and 230–250 nm (ε ~6000).[3a] The uv and nmr spectra of the monoanion of maleylacetone in water are consistent with a mixture of enol (**10a** and/or **10b**) and keto (**10c**) forms. Since only two methyl singlets could be detected at 60 MHz it is impossible at this time to determine which enol **10a** or **10b** is present in greater quantity in aqueous solution.

These results can be compared with the keto–enol equilibrium of acetylacetone. In water at ambient temperatures, there is approximately 80% of the keto form.[10] Additional conjugation might be expected to increase the quantity of enol as it does in maleylacetone.

The pK_a values of maleylacetone as determined by titration with base are 4.2 and 9.4. The first ionization constant was also determined to be 3.95 by standard spectrophotometric techniques. The difference between this value and the value obtained for pK_{a_1} by titration (4.2) is probably due to the fact that the spectrophotometric pK_a determination was carried out at a constant ionic strength while the titration was not. The uv spectra of maleylacetone between pH 2 and 6.43 show an isosbestic point at 210 nm consistent with a pH-dependent equilibrium between an acid and its anion and a pH-independent equilibrium between two or more forms of monoanion.

The ultraviolet spectra of maleylacetone at pH 0.99, 6.43, and 13 are given in the Experimental Section.

The mass spectrum (see Experimental Section) of maleylacetone is consistent with its assigned structure. A portion of its spectrum shows strong similarities to that for 4-acetonylidenebut-2-ene-4-olide (*Z*). This may be due to the loss of water in the heated inlet system or after formation of the parent (*m/e* 156) ion.

Experimental Section[11]

4-Acetonylidenebut-2-ene-4-olide (*Z*) (**8**).—To a stirred slurry of 60 g (0.45 mol) of aluminum chloride in 400 ml of methylene chloride was added 20 g (0.204 mol) of maleic anhydride. The mixture was stirred at room temperature for 30 min and 20 g (0.20 mol) of isopropenyl acetate was added over a period of ~5 min. The reaction mixture was stirred for 5 hr at room temperature, and added to 500 ml of 2 *N* HCl with sufficient ice to maintain the temperature below 10°. The dark viscous complex was decomposed by stirring. The organic phase was separated and the acid extracted with methylene chloride (total methylene chloride, 200 ml). The organic phases were combined, filtered through Celite, and washed with 200 ml of 5% Na_2CO_3 (in three portions) followed by 50 ml of saturated NaCl. The organic phase was dried with anhydrous $MgSO_4$ and evaporated at room temperature. Two recrystallizations of the yellow semisolid residue from CCl_4 (20 ml) and treatment with carbon at the last recrystallization gave 0.9–1.2 g (3.3–4.3%) of pale yellow needles of **8**: mp 79–81°; uv max (cyclohexane) 280 nm (ε 19,600); ir (KBr) 1780 (vs), 1700 (s), and 1640 cm^{-1} (s); nmr ($CDCl_3$) δ 2.58 (s, 3 H, CH_3), 5.58 (s, 1 H, vinyl), 6.46 and 7.53 (q, 2 H, J = 5.5 Hz). The mass spectrum (80 eV) showed *m/e* (rel intensity) 138 (33), 123 (100), 95 (31), and 69 (30).

Anal. Calcd for $C_7H_6O_3$: C, 60.86; H, 4.37. Found: C, 61.11; H, 3.98.

4-Acetonylidenebut-2-ene-4-olide (*E*) (**7**).—The preparation of **7** was carried out using the same quantities and procedure as used in the preparation of **8**. However, when the crude semisolid was allowed to stand at −6° for 2 days, 0.45 g (1.15%) of a light tan solid, mp 100.5–101.5° (lit.[6] mp 97–101°), is obtained after recrystallization from carbon tetrachloride. The nmr spectrum of this compound is identical with that reported by Nilsson.[6]

Maleylacetone (**4**).—To 1.274 g (0.0092 mol) of butenolide **8** was added 25 ml of 1 *N* NaOH. The solid dissolved within a few minutes to give an orange solution which was washed with 10 ml of methylene chloride. The basic phase was cooled and acidified with concentrated HCl, and rapidly extracted with 40 ml of methylene chloride. The combined organic phases were washed with 10 ml of saturated NaCl solution and dried over anhydrous $MgSO_4$. A small amount of carbon was added, the solution filtered, and the solvent removed at room temperature at reduced pressure leaving 0.512 g (36%) of a pale yellow oil, which partially solidifies when stored at −6° for several days. The nmr spectrum showed that maleylacetone is a mixture of "pseudo" acid and open acid with the ratio variable between different reactions. The nmr spectrum ($CDCl_3$) showed δ 2.30 (s, 3 H, CH_3), 2.9–3.2 (q, 2 H, J = 16 Hz, CH_2 adjacent to asymmetric center), 6.12 and 7.35 (q, 2 H, J = 5.5 Hz, ring protons), and 7–8 (broad OH) for "pseudo" acid, **4a**, and 2.24 (s, 3 H, CH_3), 5.79 (s, 1 H, C-5 vinyl), 6.38 (s, 2 H, C-2 and C-3 vinyls), and 7–8 (broad OH) for the open acid **4b**. The position of the OH signal is variable and frequently a very broad signal is observed. The nmr spectrum (benzene-d_6) showed δ 1.70 (s, 3 H, CH_3), 2.1–2.8 (q, 2 H, J = 16 Hz, CH_2 adjacent to asymmetric center), 5.70 and 6.78 (q, 2 H, J = 5.5 Hz, ring protons) due to **4a** and δ 1.52 (s, 3 H, CH_3), 4.9 (s, 1 H, C-5 vinyl), and 5.58 and 6 (q, 2 H, J = 13 Hz, C-2 and C-3 vinyls) due to **4b**. Irradiation of the downfield half of the vinyl quartet of **4a** caused the upfield half of the quartet to collapse to a singlet thus fully exposing the previously obscured quartet of **4b**. The OH protons were not obvious in benzene-d_6 probably due to its absorption occurring over a wide area of the spectrum. The nmr spectrum in H_2O (reference, H_2O taken as 5 ppm from TMS) showed δ 2.22 (s, 3 H, CH_3), 3.28 (s, 2 H, CH_2), and 6.22 and 7.5 (q, 2 H, J = 5.5 Hz, ring protons) due to **4a** which appears to be the only isomer in this solvent. The ir spectrum (neat) showed 4000–3000 (broad, OH) and 1744–1704 cm^{-1} (d, C=O's). The mass spectrum (80 eV) showed *m/e* (relative intensity) 156 (51), 138 (21), 123 (86), 111 (100), 99 (28), 95 (42), 85 (32), and 69 (31). The uv spectrum showed $\lambda_{max}^{pH\ 0.99}$ 195 nm (ε 9300); $\lambda_{max}^{pH\ 6.43}$ 312 (ε 9300), 243 (4400), 212 (4100); $\lambda_{min}^{pH\ 6.43}$ 265 (ε 3300), 225 (4000); $\lambda_{max}^{pH\ 13}$ 323 (ε 14,000), 235 (4500); $\lambda_{min}^{pH\ 13}$ 270 (ε 1600).

Anal. Calcd for $C_7H_8O_4$: C, 53.84; H, 5.16. Found: C, 53.61; H, 5.25.

Molecular distillation of the pale yellow maleylacetone at 0.5 μ at 25° afforded a colorless viscous oil with identical nmr and ir spectra as described above.

Anal. Calcd for $C_7H_8O_4$: C, 53.84; H, 5.16. Found: C, 53.47; H, 5.47.

The aqueous phase, after standing for several hours at room temperature deposited 0.234 g (16% based on **8**) of fumarylacetone (**5**): mp 162–165° (lit.[6] mp 158–160°); uv max (0.1 *N* $HClO_4$) 312 nm (ε 14,000) [lit.[12] uv max (0.1 *N* HCl) 315 nm (ε 13,500)]; nmr (acetone-d_6) 2.24 (s, 3 H, CH_3), 6.04 (s, 1 H, C-5 vinyl), 6.60 and 7.00 (q, 2 H, J = 16 Hz, C-2 and C-3 vinyls), 4–5 (broad, 2 H, OH's).

Fumarylacetone (**5**).—To 0.41 g (0.00296 mol) of butenolide **8** was added 5.5 ml of 1 *N* NaOH. After the solid had dissolved, the orange solution was acidified with concentrated HCl and heated for 3 min on a steam bath. On cooling the solution deposited 0.19 g (41%) of light tan needles, mp 162–166° (lit.[6] mp 158–160°).

Disodium Salt of Maleylacetone (**9**).—To 0.056 g (0.00041 mol) of **8** was added about a threefold excess of 1 *N* NaOH and

(10) J. Powling and H. J. Bernstein, *J. Amer. Chem. Soc.*, **73**, 4353 (1951).

(11) All melting points were determined on a Reichert melting point block and are uncorrected. Uv spectra were recorded on a Cary 14 spectrophotometer. Infrared spectra were run on a Perkin-Elmer 337 spectrophotometer and nmr spectra were taken on a Varian A-60 instrument with TMS as internal standard unless otherwise specified. The mass spectrum was recorded on a Hitachi Perkin-Elmer RMU-7 mass spectrometer and pH measurements were made on an Orion Model 801 digital pH meter standardized at pH 4 and 7. Microanalyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y.

(12) C. Kisker and D. I. Crandall, *Tetrahedron*, **19**, 701 (1963).

the nmr spectrum taken. The nmr spectrum (reference, water taken as 5 ppm from TMS) showed δ 2.38 (s, 3 H, CH_3), 5.55 (s, 1 H, C-5 vinyl), 6.34 (s, 2 H, C-2 and C-3 vinyls). The above solution was acidified with concentrated HCl and the nmr spectrum of the resulting solution was identical with that of maleylacetone in water. A similar experiment with butenolide **7** was carried out in order to show that it was also hydrolyzed to form maleylacetone.

Monosodium Salt of Maleylacetone (10).—To 0.038 g (0.00024 mol) of maleylacetone in 0.5 ml of D_2O was added 0.054 g (0.00064 mol) of $NaHCO_3$. After CO_2 evolution had ceased the nmr spectrum was taken and showed (reference, HOD taken as 5 ppm from TMS) δ 2.48 (s, 3 H, CH_3), 2.57 (s, 3 H, CH_3), 6.33 and 6.58 (q, 2 H, J = 12.5 Hz, C-2 and C-3 vinyls), 6.5 and 6.76 (q, 2 H, J = 12.5 Hz, C-2 and C-3 vinyls). The pD of this solution was 8.34. The solution from the nmr tube was diluted to 1 l., the pH adjusted to 7.99 with $NaHCO_3$ and HCl, and the uv spectrum run (λ_{max} 312 nm).

Titration of Maleylacetone.—Maleylacetone (0.16176 g, 0.001036 mol) in 20 ml of water was titrated with 0.1219 N NaOH using a glass electrode. The pH was measured after each 0.2 ml addition of base. The first neutralization equivalent required 8.40 ml of NaOH (0.001024 mol). The pK_a's were determined from the pH at addition of half an equivalent of base, the first two ionization constants being 4.2 and 9.4.

Dissociation Constants.—Nine acetic acid–sodium acetate buffer solutions containing lithium perchlorate were prepared to give solutions in which the sum of the acetic acid and sodium acetate concentrations was 0.01 M and the ionic strength was 0.1 M when diluted with a stock solution of maleylacetone. An Orion Model 801 digital pH meter standardized at pH 4 and 7 was used to determined the pH of each solution. Uv spectra were recorded on a Cary 14 spectrophotometer at 25° for each buffer solution containing 2.24×10^{-4} M maleylacetone. The reference cell contains buffer. Complete ionization was assumed at pH 6.43 (*i.e.*, $[anion]_{6.43} = [acid]_0$). Ratios of $[anion]_{pH}/[acid]_0$ were determined at 312 nm and taken as $O.D._{pH}/O.D._{6.43}$. A plot of these ratios gave $pK_a = 3.95$ when $[anion]_{pH}/[acid]_0 = 0.5$.

Registry No.—**4a**, 25517-95-7; **4b**, 25568-65-4; **7**, 25527-98-4; **8**, 25527-99-5; **9**, *cis,cis* (disodium salt), 25568-66-5; **9**, *cis,trans* (disodium salt), 25528-00-1; **10a** (monosodium salt), 25528-01-2; **10b** (monosodium salt), 25528-02-3; **10c** (monosodium salt), 25528-03-4.

Acknowledgment.—We wish to thank Dr. David Christman for recording the mass spectra.

The Prévost Reaction with 5-Substituted 5-Allylbarbituric Acids

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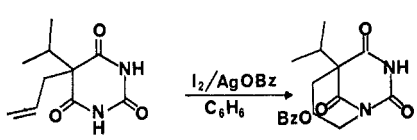
Received March 31, 1970

The Prévost reaction utilizing 5-substituted 5-allylbarbituric acids produced furopyrimidines by intramolecular O-alkylation. These compounds could be hydrolyzed readily in the presence of acid or converted to esters by treatment with the corresponding alcohol in the presence of acid.

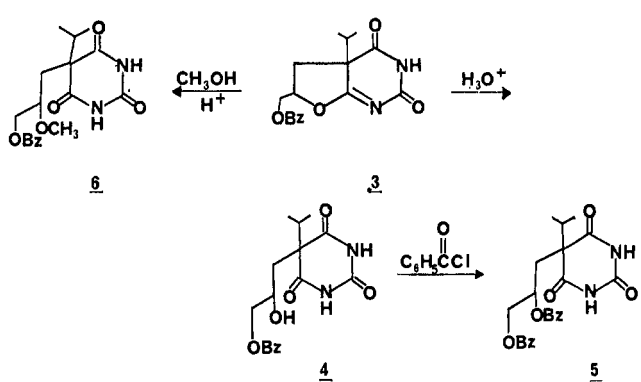
Meltzer and Lewis[2] reported the conversion of 5-isopropyl-5-allylbarbituric acid (**1**) to a bicyclic product **2** by the use of the "dry" Prévost reaction.[3]

In attempting to duplicate the work of Meltzer and Lewis in these laboratories, it was found that the product which they obtained had been assigned an erroneous structure and was not **2**, as they depicted.



1 **2**

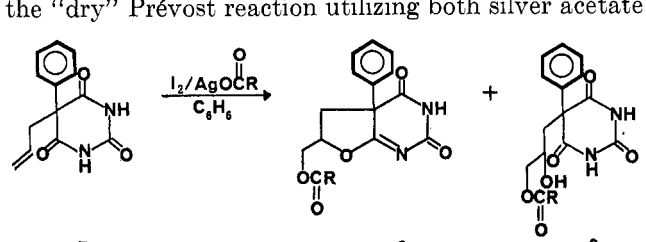
On examination, the cyclized product proved to be an O-alkylated structure, 4(a)-isopropyl-6-benzoyloxymethyl-5H,6H-furo[2,3-*d*]-$\Delta^{1,7a}$-2,4(3H)-pyrimidinedione (**3**). The infrared spectrum of **3** showed intense absorption at 1625 cm^{-1} (>C=N– stretching frequency).[4] The mass spectrum of **3** gave a molecular ion at *m/e* 330, consistent with its ascribed formula. The enol–ether, **3**, underwent facile conversion to 5-isopropyl-5-(2-hydroxy-3-benzoyloxypropyl) barbituric acid (**4**) during acid hydrolysis. The dibenzoate, **5**, was prepared by the treatment of **4** with benzoyl chloride



6 **3** **4** **5**

in pyridine. The alcohol methine proton in **4** was shifted from nmr δ 4.50 (3-proton multiplet) to 5.60 in the acylated product **5**. Compound **3** yielded a methyl ether, **6**, on treatment with methanol in the presence of acid (ether methine proton shifted 1.0 ppm downfield relative to the alcohol, **4**).

5-Phenyl-5-allylbarbituric acid (**7**) was subjected to the "dry" Prévost reaction utilizing both silver acetate



7 **8** **9**

a) R = CH_3

b) R = C_6H_5

(1) Taken in part from the dissertation presented by R. A. Robinson, July 1969, to the Graduate School of the University of Kansas in partial fulfillment of the requirements for the Doctor of Philosophy Degree.

(2) R. I. Meltzer and A. D. Lewis, *J. Org. Chem.*, **21**, 256 (1956).

(3) C. V. Wilson, *Org. React.*, **9**, 360 (1957).

(4) K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, San Francisco, Calif., 1962, p 39.