

temperature gave a mass of yellow, rather unstable crystals. Cautious recrystallization from ethanol gave approximately 5 g. of blunt yellow needles, m.p. 83.5–84.0°.

Anal. Calcd. for $C_{18}H_{20}O_2N$: C, 70.33; H, 9.5. Found: C, 70.36; H, 9.63.

Method B.¹² Reaction of Nitric Acid with 2,4,6-Tri-*t*-butylphenol.—Concentrated nitric acid (2 ml., 0.05 mole) was added to 3.93 g. (0.015 mole) of 2,4,6-tri-*t*-butylphenol dissolved in 20 ml. of glacial acetic acid. The solution was shaken under the tap and a voluminous precipitate rapidly formed. Upon filtration 3.5 g. (90%) of the nitro compound (m.p. 76–80°) was obtained. Two recrystallizations from ethanol raised the melting point to 83.5–84°. A mixture melting point with material produced by method A was 83.5–84.5°.

Regeneration of the radical and of the parent phenol upon treatment with neutral or acidic, alcoholic sodium iodide occurred exactly as described under the bromo compound.

Bis-(1,3,5-tri-*t*-butyl-2,5-cyclohexadiene-4-one). Peroxide (IV).—Upon exposure to the air, solutions of the radical rapidly turned from blue to greenish yellow. Evaporation of the solvent (usually benzene) at room temperature gave yellow crystals which, upon cautious recrystallization from ethanol, melted at 148–149° dec.

A more convenient method of preparing the peroxide consisted of oxidizing the phenol in the presence of air. In a typical run a solution of 20 g. (0.076 mole) of the phenol in 250 ml. of benzene was vigorously stirred for two days with a solution of 75 g. (0.23 mole) of potassium ferricyanide and 25 g. of potassium hydroxide in 250 ml. of water. (When air was bubbled through the reaction mixture the reaction time was reduced to a few hours.) The yellow benzene layer was separated, washed with water and evaporated to dry-

(12) University of Vermont Bachelor's Thesis, 1953, by Miss Barbara A. Johnson.

ness under an air stream at room temperature. The yellow residue was taken up in hot ethanol, filtered and quickly cooled; yield 17 g. (81%), m.p. 146–147°; after a second recrystallization m.p. 148–149°. The hot ethanolic solutions darkened rapidly and to obtain a pure product it was necessary to perform the recrystallizations rapidly.

Anal. Calcd. for $C_{28}H_{38}O_4$: C, 77.93; H, 10.54; mol. wt., 554.8. Found: C, 77.91; H, 10.65; mol. wt. (f.p., benzene), 555, 560.

Upon heating above the melting point, the peroxide decomposed to evolve isobutylene; one mole of peroxide yielding one mole of isobutylene. Thus 2.362 g. (0.00425 mole) of peroxide gave 103 ml. of gas (25°, 760 mm. or 0.0042 mole).

A detailed study of this decomposition will be reported in the near future.

The oxygen uptake of the radical was measured by oxidizing the phenol with alkaline ferricyanide in an oxygen atmosphere and measuring the oxygen absorption. Thus, 1.967 g. (0.0075 mole) of the phenol in 75 ml. of benzene and 6.35 g. (0.019 mole) of potassium ferricyanide and 1.0 g. of potassium hydroxide in 24 ml. of water were placed in a 200-ml. round-bottom flask which was attached to a 100-ml. gas buret. The flask was agitated in a mechanical shaker for 82 hours. A blank, which contained the end-point proportions of potassium ferricyanide and potassium ferrocyanide, but which excluded the peroxide, was also run. Both solutions were flushed with nitrogen prior to the run. The oxygen absorption due to the radical was 87.2 ml. at 26° and 760 mm. or 0.0036 mole. This indicates a reaction ratio of two moles of radical per mole of oxygen.

Spectra.—The ultraviolet spectra were measured in cyclohexane at concentrations of 2×10^{-4} to 5×10^{-3} mole/l. Over this range Beer's law was obeyed. The infrared spectrum of the peroxide was taken in carbon disulfide.

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[CONTRIBUTION FROM THE MOLTEANO INSTITUTE, UNIVERSITY OF CAMBRIDGE]

On the Configuration of the So-called Dihydroxymaleic Acid

By E. F. HARTREE

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Previous studies on aqueous solutions of "dihydroxymaleic" acid have indicated a *trans* configuration. Treatment of the acid with methanolic and ethanolic HCl gives 3 types of ester which are identified as *cis*, *trans* and keto forms. The ester produced by treatment with diazomethane in ethereal solution has the *trans* structure; hence the free acid appears to be dihydroxyfumaric acid. Further evidence for the proposed structures is obtained from the effect of boric acid on the spontaneous decomposition of the acid and its esters in aqueous solution.

Although "dihydroxymaleic" acid was prepared as long ago as 1874¹ and later studied extensively by Fenton,^{2–6} no satisfactory proof of its configuration has yet been given. However, the importance which this acid is now assuming in biochemistry calls for a solution of this problem, *i.e.*, whether it is dihydroxymaleic or dihydroxyfumaric acid. The evidence to be brought forward in this and in the following paper supports the view that it is dihydroxyfumaric acid (DF).

It has been shown that many plants contain a "DF-oxidase"⁷ and, by fractionating horse-radish root extracts Theorell and Swedin⁸ were able to

demonstrate a proportionality between peroxidase and DF-oxidase activities. Although the latter activity, unlike the former, was insensitive to cyanide, it was suggested that the two enzymes are identical.

In the field of photosynthesis Locke,⁹ and also Hough and Jones,¹⁰ suggested that DF was the precursor of glycol aldehyde in the synthesis of hexoses. Calvin, in a study of photosynthesis in presence of $C^{14}O_2$, showed that 6-C units were built up from pairs of 3-phosphoglyceric acid molecules. He postulates DF as an intermediate in the biosynthesis of this acid.¹¹ It is of interest that sunlight catalyzes the formation of DF from tartaric acid in presence of ferrous tartrate and air.³

Previous Work on the Structure of Dihydroxyfumaric Acid.—Fenton⁴ originally proposed the *cis* configuration for DF because the instability of its aniline salts is more reminiscent of those of

(1) E. Bourgoin, *Compt. rend.*, **79**, 1053 (1874).

(2) H. J. H. Fenton, *J. Chem. Soc.*, **65**, 899 (1894).

(3) H. J. H. Fenton, *Brit. Assoc. Advance. Sci. Rep.*, 663 (1895).

(4) H. J. H. Fenton, *J. Chem. Soc.*, **69**, 546 (1896).

(5) H. J. H. Fenton, *ibid.*, **73**, 78 (1898).

(6) H. J. H. Fenton and W. A. R. Wilks, *ibid.*, **101**, 1570 (1912).

(7) G. A. Snow and S. S. Zilva, *Biochem. J. (London)*, **32**, 1932 (1938); I. Banga and A. Szent-Györgyi, *Hoppe-Seyler's Z. physiol. Chem.*, **255**, 58 and 256 (1938); I. Banga and E. Phillipot, *ibid.*, **258**, 147 (1939).

(8) H. Theorell and B. Swedin, *Nature*, **145**, 71 (1940).

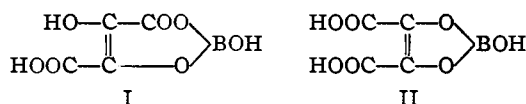
(9) A. Locke, *THIS JOURNAL*, **46**, 1246 (1924).

(10) L. Hough and J. K. N. Jones, *Nature*, **167**, 180 (1951).

(11) M. Calvin, *J. Chem. Educ.*, **26**, 639 (1949).

maleic and citraconic than those of fumaric and mesaconic acids and because he was able to prepare a diacetyl anhydride by treatment with acetyl chloride. Furthermore, by treating DF with HBr/acetic acid he obtained an isomeric form which he believed to be the *trans* isomer. The latter was also produced by reducing dihydroxytartaric acid with Zn/H₂SO₄.⁵ However, in spite of superficial differences in their crystals the two forms appeared to be chemically identical, while both darkened and charred on heating and showed no definite melting point. Fenton and Wilks⁶ were able to prepare DF by bromine oxidation of the high m.p. form of enolic oxalacetic acid. This evidence for a fumaroid structure is, however, unsound since *trans-cis* interconversions often occur under acid conditions. Furthermore, the view of Wohl and Oesterlin¹² that the high and low m.p. forms of enolic oxalacetic acid are monoxymaleic and monoxymaleic acids, respectively, is disproved by the identity of their infrared spectra.¹³

That Fenton's two acids were either identical or were rapidly converted in aqueous solutions into the one form (or into an equilibrium mixture) followed from the work of Skinner¹⁴ who found that the electrical conductivities of their solutions were identical. Further evidence of identity was obtained by Böeseken¹⁵ who studied the effect of boric acid on the conductivities. Both catechol and salicylic acid, on account of the favorable orientation of OH and COOH groups, react with boric acid in aqueous solution to produce strong acids and thus a marked enhancement of electrical conductivity above the sum of the conductivities of the components.¹⁶ It appears, therefore, that the enhancement observed in the case of DF cannot distinguish between *cis* and *trans* structures since both forms could conceivably react, I being analogous to salicylic acid and II to catechol.



Franke and Brathuhn¹⁷ compared the first and second dissociation constants of DF with those of several related dicarboxylic acids and concluded, on the basis of results summarized in Table I, that DF has the fumaroid structure.

Rothschild¹⁸ found that while maleic and citraconic acids exhibit marked chemotactic activity toward spermatozoa of bracken (*Pteridium aquilinum*), DF and fumaric and mesaconic acids are completely inactive.

Thus while the available evidence favors the *trans* configuration for DF it is based upon the properties of aqueous solutions in which keto-enol

isomerization could conceivably lead to the presence of both *cis* and *trans* forms (III, IV, V; R = H).

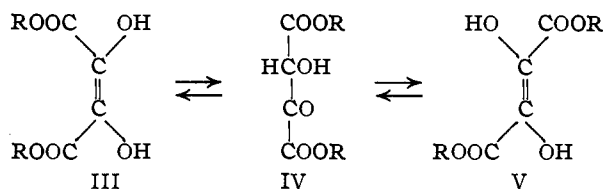


TABLE I

RATIOS OF FIRST AND SECOND DISSOCIATION CONSTANTS OF DIHYDROXYFUMARIC ACID (DF) AND OF RELATED ACIDS AS DETERMINED BY FRANKE AND BRATHUHN

	K ₁ 's	Ratio of K ₁ 's
Tartaric:succinic	15	17
DF:fumaric	9	17
DF:maleic	0.6	400
		K ₂ /K ₁
DF		75
Fumaric		58
Maleic		45,400
Mesaconic		53
Citraconic		5,100

Configuration of Esters of Dihydroxyfumaric Acid.—Although Böeseken's conductometric methods can give no evidence for the configuration of DF, it appeared quite likely that the problem could be approached by applying such methods to DF esters if the latter could be prepared without change of configuration. Fenton² described a "methyl salt" of DF which he obtained by the usual MeOH-HCl treatment. Later Fenton and Wilks⁶ showed that two isomeric ethyl esters (A and B) could be separated from a corresponding reaction mixture in EtOH and they state that two analogous methyl esters also could be prepared. Since maleic-fumaric interconversion is prone to occur in presence of mineral acid it became necessary to re-examine these esters with a view to relating them structurally to DF. The ethyl esters were obtained essentially by the method of Fenton and Wilks. Ester A, which is very soluble in ether, had m.p. 68–70° (Fenton and Wilks give 68°). In agreement with these authors this ester was found to reduce silver nitrate and cupric acetate with great rapidity and to give a transient green color with ferric chloride. The ether-insoluble ester B had m.p. 156–158° although Fenton and Wilks give 126–128°. Otherwise its properties are in agreement with those given in the original description (see Table II). A, unlike B, is very unstable and soon changes in air or in a desiccator to a yellow oil which Fenton and Wilks regarded as diethyl diketosuccinate.

Fenton was unable to decide whether the isomerism of the esters was *cis-trans* or keto-enol. The classical studies on keto-enol equilibria by Meyer¹⁹ and by Hantzsch,²⁰ however, have shown that the enol form of oxalacetic acid (monoxy-fumaric or monoxymaleic acid) shows a very pronounced absorption band between 250 and 300 mμ while the keto form shows insignificant absorp-

(19) K. H. Meyer, *Ber.*, **45**, 2860 (1912); *ibid.*, **47**, 826 (1914).(20) A. Hantzsch, *ibid.*, **48**, 1420 (1915).(12) A. Wohl and C. Oesterlin, *Ber.*, **34**, 1144 (1901).

(13) Samples of the two enol forms were prepared according to Wohl and Oesterlin. Their infrared absorption was measured by Dr. N. Sheppard to whom the author wishes to express his thanks.

(14) S. Skinner, *J. Chem. Soc.*, **78**, 483 (1898).(15) J. Böeseken, *Rec. trav. chim.*, **37**, 170 (1918); J. Böeseken and J. G. de Voogd, *ibid.*, **42**, 747 (1923).(16) J. Böeseken, *Ber.*, **46**, 2615 (1913); *Rec. trav. chim.*, **40**, 553 (1921); C. A. Zittle, *Advances in Enzymol.*, **12**, 493 (1951).(17) W. Franke and G. Brathuhn, *Ann.*, **487**, 1 (1931).(18) Lord Rothschild, *Science Progress*, **40**, 1 (1952).

TABLE II

	REAGENTS	REACTIONS OF DIHYDROXYFUMARIC ACID AND ITS ESTERS			
	AgNO ₃	Cu(OOCCH ₃) ₂	FeCl ₃	NaOH	I ₂ (in KI)
Dihydroxyfumaric acid	White ppt. darkens slowly	Bright green soln., Cu ₂ O deposited slowly	Grey-brown: violet with NaOH	Immediate decolorization
Fenton's ethyl ester B (IV, R = Et)	Slow reduction, intermediate red-brown color	Very slow reduction, Cu ₂ O deposited	Delayed decolorization
Fenton's ethyl ester A (III, R = Et)	Rapid reduction to Ag	Immediate reduction, yellow Cu ₂ O quickly turning red	Transient green	Transient yellow	Immediate decolorization
Methyl ester (V, R = Me)	Slow reduction	Slow reduction, Cu ₂ O deposited	Transient yellow	Immediate decolorization
Ascorbic acid	Rapid reduction	Immediate red-yellow ppt.	Green after NaOH	Immediate decolorization

tion in this region. Hantzsch found that on dissolution of solid oxalacetic acid in various solvents keto-enol equilibria are rapidly established, *viz.*, 0, 3, 63 and 100% enol in dilute HCl, water, ethanol and ether, respectively. A similar band is shown by ascorbic acid, which is known to have a *cis* ene-diol structure.²¹

From ultraviolet measurements it has now been shown that while A retains the ene-diol group, B does not (Fig. 1); a conclusion which is in accord with the relative rates of their reactions with I₂. The latter must therefore be diethyl ketomalate

(IV, R = Et) while the low m.p., ferric chloride reaction and powerful reducing action of A (see Raistrick and Stickings²²) suggest *cis* OH groups, *i.e.*, diethyl dihydroxymaleate (III, R = Et). The analogy between ethyl ester A and ascorbic acid (Table II) also points to a *cis* configuration. A can be converted into B by boiling with absolute ethanol while cold EtOH/HCl brings about a partial change of B into A.

The two dimethyl esters were apparently not investigated by Fenton and Wilks. Two crystalline forms appear in the reaction mixture: the first crops being bundles of leaflets and the later ones flattened needles. However, microscopic examination shows that the latter are merely elongated forms of the former; in fact the two forms have identical melting points and show the same intense ultraviolet absorption band at about 300 m μ (Fig. 1). On the basis of its stability in air, its sparing solubility in ether and its comparatively weak reducing properties the fumaroid structure (V, R = Me) is assigned to this ester.

A study of the changes with time in the ultraviolet absorption of DF and its esters in various solvents has shown that the enol forms are stable in ether whereas in aqueous or ethanolic solutions slow irreversible changes take place involving the complete disappearance of the ultraviolet band. It therefore appeared that treatment of DF with diazomethane in pure ether at a low temperature would be the most likely method of obtaining an ester with unchanged configuration. Smith²³ found that the ester so prepared at -10° in ether/ethanol was identical with the methylic salt of Fenton.² It is unlikely that any configurational change occurred in Smith's experiments but to reduce the possibility still further the esterification has been repeated in anhydrous ether at -40°. Since the product was identical with Me-ester V obtained by Fenton's method it would appear that the acid probably has the fumaroid structure.

Effect of Boric Acid on the Ultraviolet Absorption of Dihydroxyfumaric Acid and its Esters.—Unfortunately Et-ester III and Me-ester V are too insoluble in water to allow any conductometric data to be obtained by Böeseken's procedure. A

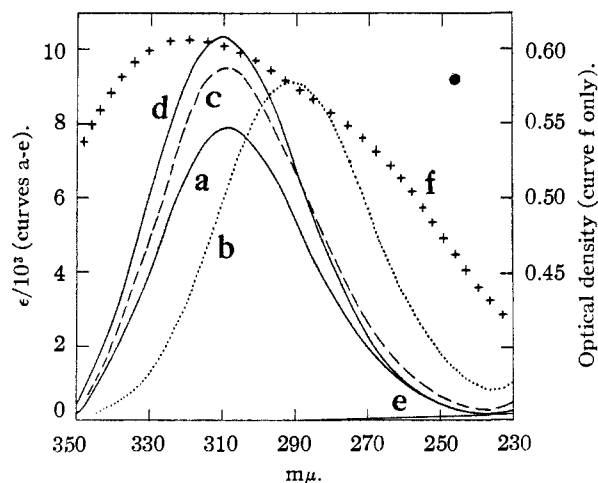


Fig. 1.—Ultraviolet absorption curves of dihydroxyfumaric acid and its esters. Measurements carried out on 10⁻⁴ M solutions in ether and O₂-free water. Curve b is approximate owing to the instability of DF in aqueous solution. ϵ = optical density of 1 cm. soln./molar concn. a, dihydroxyfumaric acid in ether; b, dihydroxyfumaric acid in water; c, Me-ester V (dimethyl dihydroxyfumarate) in ether; d, Et-ester III (diethyl dihydroxymaleate, Fenton A) in ether; e, Et-ester IV (diethyl ketomalate, Fenton B) in ether or water; f, dihydroxyfumaric acid as a dried film (from ether). [The film obtained by rapid evaporation of an aqueous ethanolic solution gave a similar curve.] Free rotation about the bond joining the α -C atoms of tartaric and succinic acids in solution will allow the carboxyl groups, on account of their mutual repulsion, to take up mean spatial positions analogous to fumaric rather than to maleic acid.

(21) R. W. Herbert, E. L. Hirst, E. G. V. Percival, R. J. W. Reynolds and F. Smith, *J. Chem. Soc.*, 1270 (1933); E. G. Cox and T. H. Goodwin, *ibid.*, 769 (1936).

(22) H. Raistrick and C. E. Stickings, *Biochem. J. (London)*, **48**, 53 (1951).

(23) F. Smith, Ph.D. dissertation, University of Birmingham (1935).

spectrophotometric study was therefore made of the effect of boric acid on the stability of solutions of DF and its esters in absence of oxygen. Since Et-ester IV showed virtually no absorption at 300 $m\mu$, the height of the band in this region shown by structures of types III and V is a convenient measure of their concentrations. Data obtained by this method are recorded in Fig. 2. It is seen that in the case of DF where a complex is known to be formed¹⁵ the slow spontaneous decomposition is checked. The instability of Me-ester V is not changed by boric acid, as would be expected if it is a *trans* ester, while the partial (50%) stabilization of the very unstable Et-ester III indicates a *cis* structure.

Failure to obtain a more complete stabilization of Et-ester III can be explained on the basis of experiments by Militzer,²⁴ who showed that while those sugar derivatives which give a positive Böeseken reaction are also protected by borate ion from oxidation by the Benedict reagent, ascorbic acid was not so protected. Although Militzer's experimental conditions are quite different from those of Böeseken the former's results indicate that ascorbic acid is an exception to the Böeseken rule. This was confirmed by a study of the influence of boric acid on the conductivity of ascorbic acid (Table III).

TABLE III

EFFECT OF BORIC ACID ON THE ELECTRICAL CONDUCTIVITY OF ASCORBIC ACID

κ = specific conductivity, $\Delta\kappa$ = conductivity of ascorbic acid in boric acid—(conductivity of ascorbic acid in water + conductivity of boric acid), boric acid, 0.5 *M*, *T* = 25°.

Ascorbic acid dilution, l./mole	Solvent	10 ⁶ κ	10 ⁶ $\Delta\kappa$
4	Boric acid	1253	—76
4	Water	1293	
16	Boric acid	664	—77
16	Water	705	
64	Boric acid	335	—59
64	Water	357	
No asc. acid	Boric acid	36	

Acidity of the Ene-Diol Groups.—Böeseken has not recorded experiments with acidic ene-diols and the experiments just described suggest that no stable complex is formed between boric acid and such structures.

DF titrates as a dibasic acid with NaOH and thymolphthalein and the non-acidic nature of the ene-diol group has been confirmed by measurements of CO₂ liberated from bicarbonate-CO₂ buffers (Table IV). Ascorbic acid liberates one equivalent of CO₂ and DF almost two equivalents while Et-

TABLE IV

CO₂ LIBERATED FROM NaHCO₃/CO₂ BUFFER BY ASCORBIC ACID, DIHYDROXYFUMARIC ACID (DF) AND DF ESTERS

	Wt. taken (mg.) (a)	CO ₂ evolved per mole a
Ascorbic acid	3.12	1.00
DF	1.25	1.91
Ethyl ester III (Fenton A)	5.11	0.74
Ethyl ester IV (Fenton B)	3.96	0.70 ^a
Methyl ester V	3.05	0.90

^a CO₂ evolved slowly.

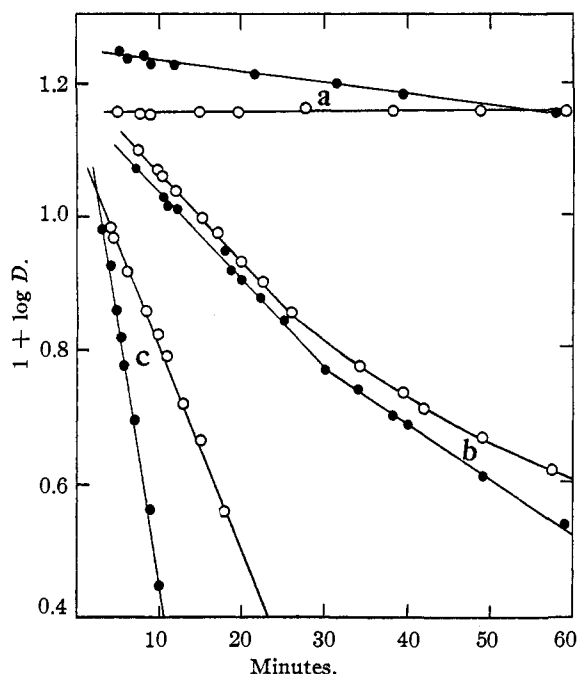


Fig. 2.—Effect of boric acid on the spontaneous decomposition of dihydroxyfumaric acid (DF) and its esters in absence of O₂ measured by changes in optical density at the absorption maxima (see Fig. 1): —●—●—, solutions in water; —○—○—, solutions in 0.25 *M* boric acid; O₂-free conditions as described in the text; solute, 2 × 10⁻⁴ *M*: a, dihydroxyfumaric acid; b, Me-ester V; c, Et-ester III.

ester III and Me-ester V liberate somewhat less than one equivalent. This deficiency probably arises from their instability (Fig. 2). Even Et-ester IV slowly liberates 0.7 equivalent of CO₂ which indicates that it is in equilibrium with an enol form in bicarbonate buffer.

It can therefore be concluded (a) that a complex between boric acid and an ester of type III would not be very stable and (b) that structures I and II are both possible and therefore no indication of the configuration of DF can be obtained by Böeseken's procedure (see discussion by Lundblad²⁵).

Evidence for the *trans* Configuration of Dihydroxyfumaric Acid.—The comparison of DF with acids of related structure in terms of dissociation constants and chemotactic properties gives evidence for a *trans* structure in aqueous solution. The formation of a grey-brown color with FeCl₃ (violet on addition of alkali) is in contrast to the customary green color expected with *cis* OH groups.

The fumaroid structure of the acid in ether solution finds support in the properties of the ester formed with diazomethane. The high stability, high m.p. and low solubilities of this ester are in marked contrast to the properties of an ethyl ester prepared by treatment with EtOH/HCl. Since both esters retain the ene-diol group they must be considered as *trans* and *cis*, respectively. The fact that two esterifications carried out under very similar conditions with ethanol/HCl and methanol/HCl yield products of different structures is perhaps due to the differing solubilities of related ethyl

(24) W. E. Militzer, *J. Biol. Chem.*, **158**, 247 (1945).

(25) G. Lundblad, *Arkiv. Kemi, Mineral., Geol.*, **25A**, No. 5 (1947).

and methyl esters in the respective reaction mixtures. Thus after ethanol/HCl treatment structures III and IV can be isolated while after methanol/HCl treatment only V (identical with that prepared with diazomethane) can be detected. Since a partial conversion of IV into III can be shown to occur under the conditions of ethylation, and since Me-ester V is not accompanied by other isomers after methylation under analogous conditions, it is reasonable to conclude that the *trans* structure is the initial product.

While unequivocal proof of the structure of solid DF can only be obtained from studies on the solid itself the evidence now presented favors the view that DF is a *trans* enol. According to Hantzsch's work on oxalacetic acid it is possible to regard ether as an enolizing solvent and water or acids as ketonizing solvents. Although oxalacetic acid goes over to the keto form almost instantaneously in water, DF remains as an enol both in ether and in water. This fact makes it unlikely that solid DF is ketomalic acid. Further evidence against the keto structure is (a) the sparing solubility of DF in water while the ketomalate analogs, oxalacetic and malic acids are very soluble (b) the demonstration of an absorption band at 310–320 $m\mu$ in a film of the solid acid (Fig. 1, f). A control experiment with a malic acid film revealed no absorption maximum within the same wave length range. There appears to be no direct evidence for the existence of the ketomalate form but it is regarded by Franke and Brathuhn¹⁷ as the source of CO₂ evolved spontaneously from aqueous DF solutions. (That CO₂ arises in this way is suggested by the work of Pedersen²⁶ who found that stabilization of the keto form in such equilibria, e.g., by the introduction of two α -Me groups into acetoacetic acid, greatly increases the rate of spontaneous decarboxylation.)

Thus while little doubt remains that DF has the *trans* structure in solution, the application of the evidence to the solid acid presupposes an absence of rapid *cis-trans* changes in ethereal solution. However, final confirmation of the structure is given in a following paper by Gupta²⁷ in which an X-ray analysis of the crystalline hydrate yields unequivocal evidence that it is indeed dihydroxyfumaric acid.

Experimental Part

In order to prevent contamination by heavy metals which catalyze the decomposition of DF all glassware was washed in chromic-sulfuric acid followed by 6 N HCl. After copious washing with tap-water the apparatus was finally rinsed with glass-distilled water. Boric acid (analytical grade) was recrystallized from glass-distilled water. Ethyl ether was redistilled from sodium; methanol and ethanol from BaO.

The liberation of CO₂ from bicarbonate buffer by DF and its esters was measured in Barcroft differential manometers at 20°. The substances in dry form were weighed out in dangling cups which were suspended from the central tubes. The flasks contained 3.3 ml. of 1.3% NaHCO₃ through which N₂ containing 5% of CO₂ had previously been passed. The manometers were filled with the same mixture by 3-fold evacuation to 50 mm.

Absorption in the ultraviolet region was measured at 18–20° with a Hilger Uvispec spectrophotometer using 1-cm. cells cleaned in concd. HNO₃ and rinsed with glass-distilled water. For the preparation of aqueous solutions, glass-

distilled water was boiled out and cooled in a stream of O₂-free N₂. The required volume of water was pipetted into a flask through which N₂ was passing and which contained sufficient solute to give 1–2 $\times 10^{-3}$ M solution. (Me-ester V and Et-ester IV were not readily wetted by water and their solution was hastened by the presence of 2% ether.) Of this solution 0.3 ml. was transferred to a spectrophotometer cell containing 2.7 ml. of O₂-free solvent covered by liquid paraffin. The same procedure was adopted when boric acid solution replaced water. Dry films of acids were prepared by evaporating 3 ml. of 0.01 M ethereal solution in a silica absorption cell by means of a stream of warm air.

Electrical conductivities were measured with a Cambridge Instrument Co. A.C. bridge.

All m.p.'s are uncorrected. Analyses were carried out by Drs. Weiler and Strauss, Oxford, except for Et-ester III which was analyzed by Mr. G. R. Purdie of the Cambridge University Chemical Laboratory.

Dihydroxyfumaric Acid.—Commercial samples were found to be heavily contaminated with iron and were thus very unstable in solution. The pure anhydrous acid was prepared by a modification²⁸ of a method due to Neuberger and Schwenk.²⁹ Crystals of the dihydrate suitable for X-ray analysis were obtained by dissolving 1 g. of anhydrous acid in 20 ml. of acetone, adding 12 ml. of water and allowing to crystallize overnight at –15°.

Ethylation with EtOH/HCl.—A solution of 3.00 g. of DF in 120 ml. of ethanol was saturated with dry HCl at 0°. The resulting ppt. of DF redissolved on addition of 120 ml. of ethanol. After standing 4 days at room temperature the solution was evaporated *in vacuo* to a small bulk when crystals (mostly needles) appeared. The mixture was filtered at 0° and the filtrate treated with 2 vol. of ice-water. The resulting crystals were collected on the same filter and air-dried (1.46 g.). Dry ether (20 ml.) was passed through the filter when the needle crystals dissolved. The residue on the filter (0.44 g.), consisting of short prisms, was Fenton's ester B, diethyl ketomalate (Et-ester IV). After crystallization from ethanol-light petroleum it had m.p. 156–158°. It showed no absorption at 300 $m\mu$.

Anal. Calcd. for C₈H₁₂O₆: C, 47.0; H, 5.9. Found: C, 47.2; H, 6.1.

The ether washings contained 1.02 g. of Fenton's ester A, diethyl dihydroxymaleate (Et-ester III) which crystallized slowly as long needles from the Na₂SO₄-dried solution at –15° (m.p. 58–60°). If left in contact with air this ester formed a monohydrate.

Anal. Calcd. for C₈H₁₂O₆·H₂O: C, 43.1; H, 6.3. Found: C, 43.1; H, 6.1.

When left overnight in a vacuum desiccator the ester changed into a yellow oil. Analysis of the anhydrous ester was therefore attempted immediately after 1 hr. in a vacuum desiccator containing silica gel.

Anal. Calcd. for C₈H₁₂O₆: C, 47.0; H, 5.9. Found: C, 46.0; H, 5.7; $\epsilon_{\text{max}}^{310}$ (in ether) 10.4 $\times 10^3$.

Methylation with MeOH/HCl.—A solution of 3.00 g. of DF in 30 ml. of absolute methanol was saturated with dry HCl at 0° and diluted with 30 ml. of methanol. Crystallization began almost at once and within 6 hr. copious bundles of leaflets had appeared in the solution. This material was filtered off and successive crops collected at 24 hr. (leaflets and flattened needles) and at 72 hr. (needles only); total yield 1.61 g. of dimethyl dihydroxyfumarate (Me-ester V). The m.p.'s of these fractions varied from 175–177° to 183–184° and their ultraviolet absorption spectra were identical ($\epsilon_{\text{max}}^{309}$ 9.5 \pm 0.1 $\times 10^3$). The fractions could not be differentiated by the reactions of Table II and crystallization from MeOH, in which the ester is sparingly soluble, yielded in all cases a characteristic mixture of slender oblique-ended prisms and twinned square plates, m.p. 181–184°, unchanged on further recrystallization.

Anal. Calcd. for C₆H₈O₆: C, 40.9; H, 4.55. Found: C, 40.9; H, 4.56.

Further small crops were obtained by adding water to the 72-hr. filtrate and by concentrating the later mother liquors. In all cases only Me-ester V could be isolated; no detectable quantities of an ether-soluble component analogous to Et-ester III were present.

(26) K. J. Pedersen, *THIS JOURNAL*, **51**, 2098 (1929).

(27) M. P. Gupta, *ibid.*, **75**, 6312 (1953).

(28) E. E. Snell, "Biochemical Preparations," Vol. III, in press.

(29) C. Neuberger and E. Schwenk, *Biochem. Z.*, **71**, 112 (1915)

Methylation with Diazomethane.—A solution of 1.00 g. of DF in 250 ml. of ether was cooled to -40° and treated with a dried (KOH pellets) ethereal solution of 0.58 g. (2.04 moles) of diazomethane. A white flocculent precipitate appeared almost at once. The reaction mixture was evaporated *in vacuo* to 100 ml. and the solid (0.37 g.) filtered off. Evaporation to 50 ml. yielded further material (0.10 g.) which gave a faint DF reaction with FeCl_3 . The first

crop showed all the reactions of the methyl ester prepared by the previous method and when recrystallized from MeOH it yielded the same characteristic mixture of crystal types; m.p. $178-180^\circ$, mixed m.p. with previous sample $178-180^\circ$.

Anal. Calcd. for $\text{C}_6\text{H}_8\text{O}_6$: C, 40.9; H, 4.55. Found: C, 40.5; H, 4.55.

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[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

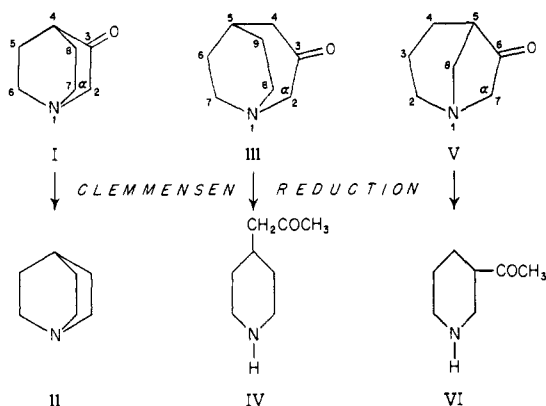
Rearrangement of α -Aminoketones During Clemmensen Reduction. XI. The Reduction of Atom-bridged Bicyclic α -Aminoketones^{1,2}

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Unlike 3-ketoquinuclidine, which undergoes no C_α -N bond cleavage on Clemmensen reduction, two related atom-bridged bicyclic α -aminoketones, 3-keto-1-azabicyclo[3.2.2]nonane and 6-keto-1-azabicyclo[3.2.1]octane, were found to give ring-cleaved reduction products. Thus, the former yielded some 4-piperidylacetone and the latter, 3-acetyl-piperidine under Clemmensen reduction conditions.

An exception to cleavage of the C_α -N bond which has been found to occur generally during the Clemmensen reduction of an α -aminoketone¹ lies in the behavior of 3-ketoquinuclidine (I), which is converted to quinuclidine (II).^{3,4} Since compound I is also the only example of an α -aminoketone of the atom-bridged bicyclic type which has been investigated with respect to its Clemmensen reduction product, it was of interest to determine whether the exceptional behavior, *i.e.*, non-cleavage of the C_α -N bond, was exhibited by other atom-bridged bicyclic α -aminoketones. 3-Keto-1-azabicyclo[3.2.2]nonane (III) and 6-keto-1-azabicyclo[3.2.1]octane (V) were selected as examples for study, the first as a ring homolog of I and the second as a ring isomer of I.



The synthesis of 3-keto-1-azabicyclo[3.2.2]nonane was modeled after the Clemo and Metcalfe method for 3-ketoquinuclidine,³ but difficulty was encountered in the Dieckmann closure of the second

(1) For article X in this series, see N. J. Leonard, G. Fuller and H. L. Dryden, Jr., *THIS JOURNAL*, **75**, 3727 (1953).

(2) This work was supported in part by a grant from E. I. du Pont de Nemours and Company, Inc.

(3) G. R. Clemo and T. P. Metcalfe, *J. Chem. Soc.*, 1989 (1937).

(4) Dr. S. H. Pines, in this Laboratory, has determined the absence of any secondary amine (C_α -N cleavage) and also of 7-methyl-1-azabicyclo[2.2.1]heptane (C_α -N cleavage + rearrangement) in the reduction product.

ring. The quaternary salt from ethyl 4-pyridylacetate and ethyl bromoacetate was hydrogenated in aqueous acetic acid in the presence of platinum to give diethyl piperidyl-1,4-diacetate. The ring closure of this diester was effected in less than 1% yield, even under the most favorable conditions: potassium *t*-butoxide in xylene at high dilution under nitrogen.⁵ Following hydrolysis and decarboxylation steps, an aminoketone was obtained which was assigned structure III on the basis of analysis, infrared spectrum and the structures of its precursors. 6-Keto-1-azabicyclo[3.2.1]octane (V) was synthesized by the method of Sternbach and Kaiser.⁶

The Clemmensen reduction of 3-keto-1-azabicyclo[3.2.2]nonane (III) was carried out under the usual conditions, and the only isolable product was obtained as a picrate, m.p. $141.5-143^\circ$, with a composition satisfying the molecular formula $\text{C}_{14}\text{H}_{18}\text{N}_4\text{O}_8$. The nature of the oxygen atom in the base was revealed as that of a ketone carbonyl group by the infrared absorption peak at 1708 cm^{-1} . The infrared data, together with the melting point of the picrate, precluded the possibility of this product being 1-azabicyclo[3.2.2]nonane ($\text{C}=\text{O}$ reduction, no C_α -N cleavage: picrate, m.p. $288-289^\circ$),⁷ 2-methylquinuclidine (cleavage + rearrangement: picrate, m.p. $282-283^\circ$)⁸ or 4-*n*-propylpiperidine (cleavage + $\text{C}=\text{O}$ reduction: picrate, m.p. $108-109^\circ$),⁸ and suggested that some 4-piperidylacetone (IV) had been formed by the incomplete reduction of III. An authentic sample of 4-piperidylacetone, made by the mixed Claisen condensation of ethyl 1-benzoyl-4-piperidylacetate and ethyl acetate, followed by hydrolysis and decarboxylation, was converted to the picrate, m.p. $146-147^\circ$. A comparison of the two picrates indicated that the sample from the Clemmensen reduction product contained some impurity along with the 4-piperidylacetone picrate, but the very

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(6) L. H. Sternbach and S. Kaiser, *ibid.*, **74**, 2215 (1952).

(7) V. Prelog and E. Cerkovnikov, *Ann.*, **532**, 83 (1937).

(8) S. Wawzonek, M. F. Nelson, Jr., and P. J. Thelen, *THIS JOURNAL*, **73**, 2806 (1951).