1076.

Pressman, B. C. (1965b), Fifth Annual Meeting of the American Society for Cell Biology, Nov 10–12, Philadelphia, Pa.

Pressman, B. C. (1966), *Methods Enzymol.* (in press).

Pressman, B. C., and Park, J. K. (1963), *Biochem. Biophys. Res. Commun. 11*, 182.

Rasmussen, H., Chance, B., and Ogata, O. (1965), *Proc. Natl. Acad. Sci. U. S. 53*, 1069.

Rasmussen, H., Fischer, J., and Arnaud, C. (1964), *Proc. Natl. Acad. Sci. U. S. 52*, 1198.

Robertson, R. N. (1960), *Biol. Rev. 35*, 231.

Rossi, C. S., and Lehninger, A. L. (1964), *J. Biol. Chem. 239*, 3971.

Saris, N. E. (1963), *Soc. Sci. Fennica, Commentationes Phys. Math. 28, II*, 1.

Schneider, W. C. (1948), *J. Biol. Chem. 176*, 259.

Solomon, A. K. (1952), *J. Gen. Phys. 36*, 57.

Wähler, B. E., and Wollenberger, A. (1958), *Biochem. Z. 329*, 508.

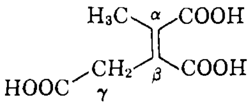
Werkheiser, W. C., and Bartley, W. (1956), *Biochem. J. 64*, 250.

α-Methyl-*cis*-aconitic Acid, *cis*-Aconitase Substrate. I. Synthesis*

Oscar Gawron and Kishan Paul Mahajan†

ABSTRACT: The synthesis of α-methyl-*cis*-aconitic anhydride, a ready source of the new aconitase substrate, α-methyl-*cis*-aconitic acid, is reported herein. Synthesis proceeds *via* condensation of ethyl cyanoacetate with diethyl methyloxalacetate, hydrolysis and decarboxylation of the resulting unsaturated cyano ester to a methylaconitic acid mixture, formation of an intermolecular anhydride from the methylaconitic acids, and partial hydrolysis and isomerization of the dianhydride to give α-methyl-*cis*-aconitic anhydride. The structure of α-methyl-*cis*-aconitic anhydride is deduced from the nmr spectrum and from formation of a complex with hydroquinone. Related compounds and related isomerizations, including a facile thermal isomerization of *trans*-aconitic anhydride to *cis*-aconitic anhydride, are described.

Investigations of substrate structural requirements for *cis*-aconitase (EC 4.2.1.3 aconitate hydratase) having been limited (Dickman, 1961) to geometrical and optical isomers, esters, and fluoro derivatives of the natural substrates, it was deemed of interest to investigate the effect of alteration of the carbon skeleton on substrate activity. As an initial attempt in this direction, the synthesis of α-methyl-*cis*-aconitic acid[1] was undertaken and in this paper and the accompanying paper (Gawron and Mahajan, 1966) the synthesis and substrate properties of the compound are reported. Interest in a methyl substituent was also stimulated by the fact that several α-methyl-substituted dicarboxylic acids are active substrates; L-methylsuccinic acid for succinic dehydrogenase (Gawron *et al.*, 1962), its oxidation product, methylfumaric acid (mesaconic acid), for L-*threo*-3-methylaspartate ammonia-lyase (EC 4.3.1.2) (Barker *et al.*, 1959), and the amination product, L-*threo*-3-methylaspartic acid, for L-*threo*-3-methylaspartate carboxyaminomethylmutase (EC 5.4.99.1) (Barker *et al.*, 1958).



α-methyl-*cis*-aconitic acid (I)

The synthesis of *cis*-aconitic acid (Malachowski and Maslowski, 1928; Malachowski *et al.*, 1928) proceeds through the synthesis of *cis*-aconitic anhydride, a compound sufficiently stable for unequivocal structure proof and for utility as a source of the relatively unstable *cis*-aconitic acid. By analogy, the synthesis of α-methyl-*cis*-aconitic acid (I) was directed to the synthesis of α-methyl-*cis*-aconitic anhydride (V, Figure 1). Figure 1 gives the sequence of reactions by which a directed synthesis of α-methyl-*cis*-aconitic anhydride was achieved. The brackets about II–IV in Figure 1 are used to indicate that the given structure is but one of several possible structures for the substance in

---

* From the Department of Chemistry, Duquesne University, Pittsburgh, Pennsylvania. *Received February 25, 1966.* This work was supported by a research grant (GM-06245) from the National Institutes of Health, U. S. Public Health Service. A preliminary report of this work has been presented (Gawron and Mahajan, 1965).

† Abstracted in part from the Ph.D. thesis of K. P. M., Aug 1965.

[1] The nomenclature of Rogerson and Thorpe (1906) for this compound has been revised to correspond with one of the products, α-methylisocitric acid, obtained by *cis*-aconitase-catalyzed hydration. The compound has previously been referred to as γ-methyl-*cis*-aconitic acid (Gawron and Mahajan, 1965).

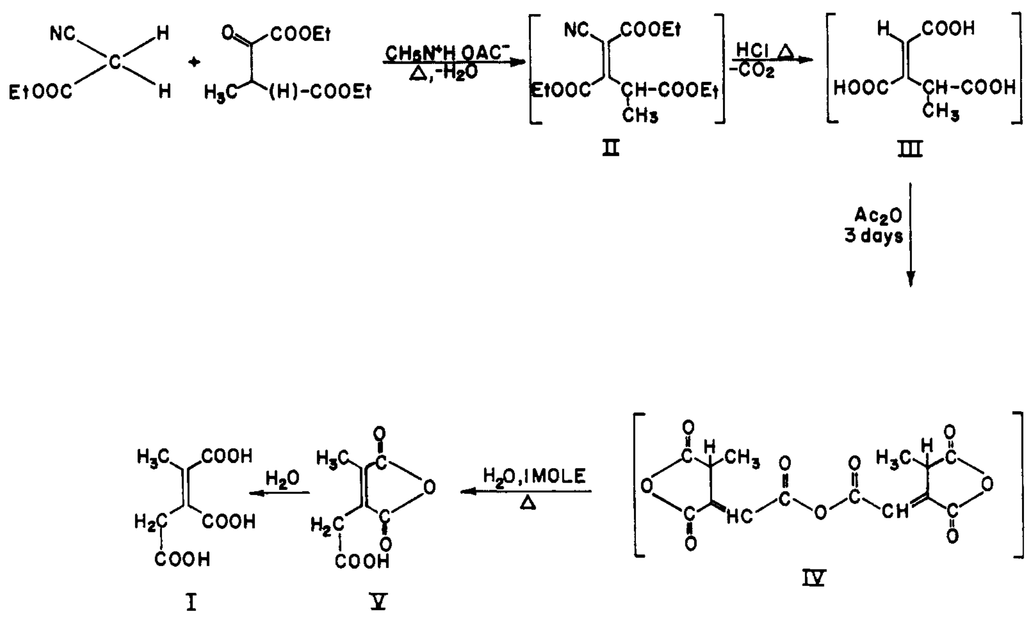


FIGURE 1: Synthetic route to α-methyl-*cis*-aconitic anhydride.

question and that assignment is not possible because the material in question is a mixture of isomers or because evidence for unequivocal structural assignment is not available.

Experimental Section

Synthetic intermediates and organic solvents were purified by distillation prior to use. If necessary, they were dried with the appropriate drying agent prior to distillation. Qualitative testing for the maleic anhydride structure was carried out as follows. Anhydride (2–3 mg) was dissolved in 0.2 ml of freshly distilled tetrahydrofuran and to this solution 1 drop of a 5% solution of hydroquinone in tetrahydrofuran was added. A positive test was indicated by the immediate appearance of yellow or green-yellow. Nmr[2] spectra were taken with a Varian A-60 spectrometer.

Melting points and boiling points are uncorrected. Microanalyses were performed by A. Bernhardt, Microanalytical Laboratory, Max Planck Institute, Germany.

Triethyl α- or γ-Cyano-γ- or α-methylaconitate (II). A mixture of 81 g (0.4 mole) of diethyl methyloxalacetate (Cox and McElvain, 1937), 81 g (0.72 mole) of ethyl cyanoacetate, 9 g (0.11 mole) of dry pyridine, 6.6 g (0.11 mole) of glacial acetic acid, and 150 ml of dry benzene was refluxed for 15 hr in a Dean-Stark apparatus. Over this period 7.2 ml (0.4 mole) of water separated. After cooling and addition of 150 ml of benzene, the benzene solution was washed successively with 0.5 M hydrochloric acid, water, 5% sodium bicarbonate, and water, 40 ml of each wash liquid being used. After drying over anhydrous sodium sulfate, benzene was removed by distillation *in vacuo*. The residual oil was then distilled *in vacuo* and the fraction (103 g, 88%), bp 137–141° (0.4 mm), was collected.

Anal. Calcd for $C_{14}H_{19}NO_6$: C, 56.56; H, 6.40; N, 4.72. Found: C, 56.56; H, 6.42; N, 4.90.

Reaction of 0.25 mole of diethyl methyloxalacetate and 0.3 mole of ethyl cyanoacetate in 140 ml of anhydrous benzene with 0.10 mole of ammonium acetate added over a 16-hr reflux period resulted in the collection of 0.23 mole of water and a 40% yield of redistilled ester.

α- or γ-Methylaconitic Acid (III). A mixture of 100 g (0.34 mole) of the cyano ester II and 500 ml of concentrated hydrochloric acid was refluxed (24 hr) until carbon dioxide evolution ceased. The reaction mixture was then cooled and washed with 50 ml of benzene. After saturation of the aqueous layer with sodium chloride, the organic acid was removed by extraction with four 400-ml portions of ether. The combined ether extracts were then dried over anhydrous sodium sulfate. Removal of ether by distillation left an orange-yellow gum from which traces of solvent were removed by storage in a vacuum desiccator over phosphorus pentoxide. All attempts at obtaining crystalline material from this gum proved fruitless and the material was used as such in the subsequent step.

Bis(methylaconitic Anhydride) Anhydride (IV). The procedure of Malachowski and Maslowski (1928) and Malachowski *et al.* (1928) for the preparation of *cis*-aconitic anhydride was applied to the preparation of this compound. A mixture of 62 g (0.33 mole) of acid III and 60 g (0.59 mole) of freshly distilled acetic anhydride was allowed to stand with occasional shaking in a stoppered flask at room temperature for 3 days. Unreacted acetic anhydride and formed acetic

---

[2] Abbreviations used: nmr, nuclear magnetic resonance.

acid were removed from the dark reaction mixture by distillation *in vacuo* at 40°, followed by 24 hr of storage in a vacuum desiccator over phosphorus pentoxide and potassium hydroxide. The partially crystalline product was then dissolved in 100 ml of dry benzene by gentle warming and allowed to crystallize for 24 hr at room temperature. The product was rapidly filtered and washed with 50 ml of dry benzene. After drying *in vacuo*, 23 g (43%) of yellowish crystals, mp 117–122°, was obtained. The crude anhydride was further purified by continuous extraction (Soxhlet apparatus) with dry benzene, the product, 21 g, mp 121–123°, crystallizing on cooling the extract. Final purification was attained by crystallization from dry ethyl acetate, a small amount of charcoal being used to remove color. Pure product (18 g, 34%), mp 126–127°, was thus obtained. This anhydride gave a negative reaction with hydroquinone.

Anal. Calcd for $C_{14}H_{10}O_9$: C, 52.16; H, 3.11; Found: C, 52.08; H, 3.05.

α-Methyl-cis-aconitic Anhydride (*V*). A mixture of 1.24 g (3.8 mmoles) of IV, 0.069 g (3.8 mmoles) of distilled water, and 20 ml of anhydrous benzene was refluxed for 16 hr, a calcium chloride drying tube being provided for protection against atmospheric moisture. Cooling of the reaction mixture resulted in the separation of an oil which was induced to crystallize by freezing the reaction mixture in a Dry Ice–acetone bath followed by thawing at room temperature. On filtering and drying *in vacuo*, 1.3 g (100%), mp 51.5–52.5°, of α-methyl-*cis*-aconitic anhydride was obtained.

Anal. Calcd for $C_7H_6O_5$: C, 49.41; H, 3.53; Found: C, 49.49; H, 3.57.

Thermal Conversion of trans-Aconitic Anhydride to cis-Aconitic Anhydride. A mixture of 15.6 g (0.1 mole) of *trans*-aconitic anhydride, mp 135–140° (Malachowski *et al.*, 1928), and 100 ml of dry benzene was refluxed (with protection from atmospheric moisture) for 10 hr. On cooling, *cis*-aconitic anhydride crystallized from the benzene solution. Rapid filtration and drying *in vacuo* yielded 14 g (90%), mp 74–75°, lit. (Malachowski and Maslowski, 1928; Malachowski *et al.*, 1928) mp 74°, of *cis*-aconitic anhydride.

Triethyl α-Methylaconitate (*VI*). To a 500-ml two-necked round-bottom flask, fitted with a dropping funnel and a Dean-Stark trap, were added 35 g (0.18 mole) of methylaconitic acid (III), 46 g (1.0 mole) of absolute ethanol, 0.2 g of *p*-toluenesulfonic acid, 1.0 ml of concentrated sulfuric acid, and 200 ml of anhydrous toluene. The reaction mixture was then heated to rapid reflux and the refluxing was continued for 10 hr. At 0.5-hr intervals during this period, the Dean-Stark trap was tapped and simultaneously 25 ml of a mixture of 69 g (1.5 mole) of absolute ethanol and 500 ml of anhydrous toluene was added to the flask. At completion of the reaction, the hot mixture was poured into an ice–water mixture. The organic layer was separated and the aqueous layer was extracted with two 200-ml portions of ether. The toluene and ether extracts were combined, washed with 50 ml of 5% sodium bicarbonate and 100 ml of water, dried over anhydrous sodium sulfate, and then concentrated by distillation *in vacuo*. The residue on vacuum distillation yielded 45 g (90%) of triethyl α-methylaconitate, bp 112–117° (0.3 mm).

Isomerization of Triethyl α-Methylaconitate (*VI*) *to Triethyl α-Methylaconitate* (*VII*). Isomerization of triester VI to triester VII was achieved by the procedure of Birch *et al.* (1923). A mixture of 42 g (0.16 mole) of triester VI, 60 g (0.44 mole) of potassium hydrogen sulfate, and 5 ml of water was refluxed (oil bath temperature 110–120°) for 5 hr. The reaction mixture was then cooled, 100 ml of water was added, and the separated oil was extracted with 200 ml of benzene. The benzene extract was washed with bicarbonate and water and then dried over anhydrous sodium sulfate. After removal of benzene by distillation, the product was distilled and the fraction, 26 g (62%), bp 142° (2 mm), was collected.

Anal. Calcd for $C_{13}H_{20}O_6$: C, 57.35; H, 7.35. Found: C, 57.20; H, 7.36.

Methylaconitic Acid (*VIII*). Triethyl α-methylaconitate (VII) (45 g, 0.16 mole) was refluxed with 250 ml of concentrated hydrochloric acid for 20 hr. After cooling, the reaction mixture was saturated with sodium chloride and then extracted with 200-ml portions of ether. The combined extracts were dried with anhydrous sodium sulfate and then evaporated at 40° to yield a gummy noncrystalline residue. On storage for 10 days *in vacuo* over potassium hydroxide and phosphorus pentoxide, the material failed to crystallize. Crystalline material was obtained by trituration of the gum with 30 ml of cold, concentrated hydrochloric acid followed by storage at 5° for 4 hr. The crystals were separated by filtration, washed with 5.0 ml of concentrated hydrochloric acid, and then dried *in vacuo*. The acid, 6.0 g, thus obtained melted over a wide range, 130–164°. It was purified by two recrystallizations from ethyl acetate–petroleum ether (bp 40–60°), 3.5 g of analytically pure acid, mp 179–181°, being obtained.

Anal. Calcd for $C_7H_8O_6$: C, 44.68; H, 4.26. Found: C, 44.70; H, 4.29.

Bis(*methylaconitic Anhydride*) *Anhydride* (*IX*). A mixture of 14 g (0.075 mole) of gummy methylaconitic acid VIII and 12.2 g (0.12 mole) of freshly distilled acetic anhydride was kept with occasional shaking at room temperature for 72 hr. Unreacted anhydride and acetic acid were removed *in vacuo* at 40°. The residue, an orange gum, was stored *in vacuo* over phosphorus pentoxide and potassium hydroxide for 24 hr to remove traces of solvent. During this period the gum crystallized. The crystalline material was suspended in 25 ml of dry benzene, filtered rapidly, and dried *in vacuo*. Anhydride (6 g, 50%), mp 117.5–118.5°, giving a positive hydroquinone test, was obtained. The crystalline acid also yielded the same anhydride.

Anal. Calcd for $C_{14}H_{10}O_9$: C, 52.16; H, 3.11. Found: C, 52.11; H, 3.21.

Methylaconitic acid VIII, crude gum, was also converted to a bisanhydride by refluxing with acetyl chloride. On work-up and recrystallization, 62% of anhydride, mp 122–124°, positive in the hydroquinone test, was obtained.

Results and Discussion

Synthetic routes to compounds isomeric with a methyl-*cis*-aconitic acid have been set forth by Rogerson and Thorpe (1906) and by Anschutz and Deschauer (1906). The former investigators obtained by saponification and decarboxylation an acid, mp 159°, from the condensation product of diethyl methyloxalacetate with cyanoacetate and the same acid from the methylated condensation product of diethyl oxalacetate and ethyl cyanoacetate.[3] The latter investigators obtained a different acid, mp 169–172°, by saponification and decarboxylation of 1,1,2,3,3-pentacarbomethoxybutene-1. Rogerson and Thorpe (1906) did not attempt to write an unequivocal structure for their acid but Anschutz and Deschauer (1906) wrote the γ-methyl-*cis*-aconitic acid structure for their acid, albeit neither acid could, of course, be structurally characterized at the time. However problematical the structure of these acids, it was felt that they could serve as a starting point for the synthesis of the desired anhydride and accordingly attention was turned to the synthesis of the acid melting at 159°. The initial step in this synthesis as described by Rogerson and Thorpe (1906) is a sodium ethoxide catalyzed condensation of ethyl cyanoacetate with diethyl methyloxalacetate to yield a triethyl cyano ester. However, neither in this laboratory nor in other laboratories (Desai, 1932; R. Bentley, 1965, personal communication) could the initial condensation be practically effected with sodium ethoxide, and so other catalysts were sought. While Desai (1932) demonstrated that potassium ethoxide gave a 20% yield of cyano triester, it was felt that catalysts of the type employed in Cope–Knoevenagel condensations would be more effective, particularly so, since ammonium acetate has been very successfully employed (Cragoe *et al.*, 1950; Kessar *et al.*, 1961, 1962) in similar condensations. Accordingly, ammonium acetate and pyridinium acetate were investigated, both catalysts being used with continuous removal, by distillation with benzene, of the water formed in the reaction.

As reported in the Experimental Section both catalysts effected the condensation of ethyl cyanoacetate with diethyl methyloxalacetate, ammonium acetate giving a 40% yield of desired ester and pyridinium acetate a substantially higher yield, 88%. The obvious advantage of these catalysts is presumably due to their concerted acid–base mechanism of action in effecting the condensation (Hein *et al.*, 1961). It should, however, be pointed out that effectiveness of these catalysts may also be due to catalysis of the dehydration reaction subsequent to catalysis of the initial condensation. A rapid dehydration followed by rapid removal of water by benzene distillation would prevent reversal of the initial condensation, thus ensuring completion of the reaction. Mechanistically, the dehydration would also be catalyzed by the same catalyst, the catalyst acting in a concerted fashion.

Location of the double bond of the isolated cyano triester was not attempted; the product is seemingly a mixture of double bond isomers, the nmr spectrum being complex, and is thus similar to the product obtained by Desai (1932), ozonolysis of the latter giving evidence for α,β and β,γ double bonds.

Saponification and decarboxylation of cyano triester II yielded a gummy acid mixture, III, from which crystalline material could not be obtained. The crude acid was, therefore, used as such for conversion to anhydride IV by treatment with acetic anhydride. Anhydride IV is seemingly a bisanhydride, the analytical data and its conversion to α-methyl-*cis*-aconitic anhydride (V) by partial hydrolysis and thermal isomerization substantiating this conclusion. The structure of IV is, however, not readily assignable since it lacks the maleic anhydride ring system (negative hydroquinone test) and is not sufficiently soluble in the usual nmr solvents to give a distinct nmr spectrum. Differentiation between the maleic anhydride ring system and other anhydride rings by color formation with hydroquinone (Table I) was first noted by Pfeiffer and Bottler (1918) and later used by Malachowski and co-workers (Malachowski and Maslowski, 1928; Malachowski *et al.*, 1928) for differentiation between *cis*-aconitic anhydride and *trans*-aconitic anhydride. *cis*-Aconitic anhydride and maleic anhydride form 1:1 complexes with hydroquinone (O. Gawron and M. Gan, 1964; unpublished data) and presumably so do other positive reacting anhydrides. Complex forma-

TABLE I: Anhydride Complex Formation with Hydroquinone.[a]

Anhydride	Complex Formation
Maleic anhydride[b]	+
Citraconic anhydride[b]	+
Itaconic anhydride[b]	−
cis-Aconitic anhydride[c]	+
trans-Aconitic anhydride[c]	−
α-Methyl-*cis*-aconitic anhydride	+
Anhydride IV	−
Anhydride IX	+
Dichloromaleic anhydride	+
Glutaconic anhydride[d]	−

[a] Details of the test are given in the Experimental Section. [b] Pfeiffer and Bottler (1918). [c] Malachowski and Maslowski (1928); Malachowski *et al.* (1928). [d] Prepared by the procedure of Malachowski (1929).

---

[3] Rogerson and Thorpe assumed their methyl cyano esters were isomeric and that the one obtained by subsequent methylation was the triester of γ-methyl-γ-cyano-*cis*-aconitic acid.

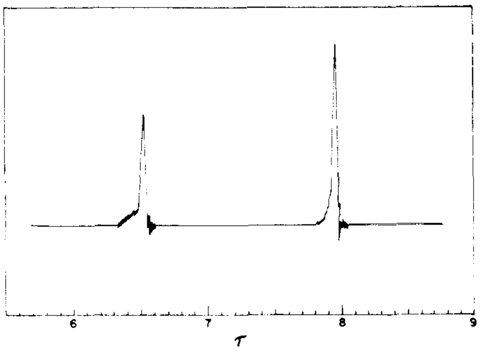


FIGURE 2: Nmr spectrum of α-methyl-*cis*-aconitic anhydride in tetrahydrofuran-d_8.

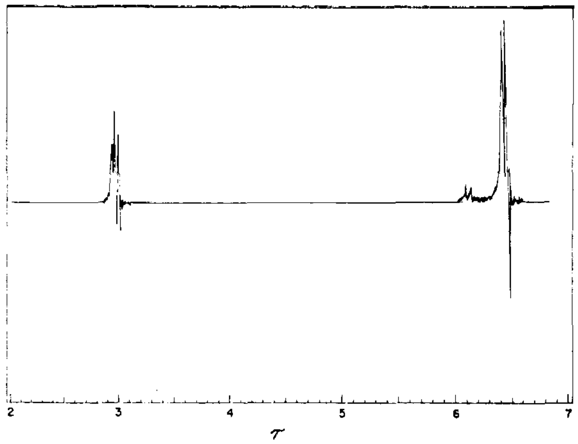


FIGURE 3: Nmr spectrum of *cis*-aconitic anhydride in tetrahydrofuran-d_8.

tion between the maleic anhydride ring and hydroquinone is analogous to quinhydrone formation and presumably these are charge transfer complexes. The absence of a maleic anhydride ring in anhydride IV suggests that the anhydride rings are either six-membered rings of the *trans*-aconitic anhydride type or five-membered rings of the itaconic anhydride type or a combination of both.

The structure of α-methyl-*cis*-aconitic anhydride (V), readily obtainable from bisanhydride IV, is apparent from the presence of a maleic anhydride ring system (positive hydroquinone test) in the compound and the nmr spectrum (Figure 2) of the compound. The spectrum shows two groups of protons, uncoupled, of the expected relative areas, three to two, with τ values, 7.95 and 6.52, expected (Jackman, 1959) for an allylic methyl group (7.9–8.1) and for a methylene group (6.2–6.8) adjacent to a double bond. It is of interest to compare the nmr spectrum of α-methyl-*cis*-aconitic anhydride with that of *cis*-aconitic anhydride (Figure 3) itself. The spectrum of *cis*-aconitic anhydride shows a narrow doublet centered at τ 6.48 and a narrow triplet at τ 2.97. The peak at τ 6.48 is due to the methylene group and is very close to the value of the methylene group of α-methyl-*cis*-aconitic anhydride. The methyl signal at τ 7.95 is, of course, absent in the spectrum of *cis*-aconitic anhydride, it being replaced by the methinyl proton peak at τ 2.97. Both the methinyl proton peak and the peak for the methylene protons show the expected splitting.

The mechanism of transformation of anhydride IV to α-methyl-*cis*-aconitic anhydride (V) is of interest since it involves in addition to an anhydride breaking step (partial hydrolysis) an isomerization of a nonmaleic anhydride system to a maleic anhydride ring system. Without certain knowledge of structure of anhydride IV mechanistic pathways, of course, cannot be discussed. However, the facile transformation, discovered in the course of this work, of *trans*-aconitic anhydride to *cis*-aconitic anhydride by refluxing in benzene may well serve as a model. This transformation (Figure 4) of *trans*-aconitic anhydride may be visualized as proceeding through anhydride ring opening with another molecule of *trans*-aconitic anhydride to give a mixed anhydride 1, followed by an allylic shift of the double bond to give the maleic acid structure 2, and finally anhydride formation by displacement to give *cis*-aconitic anhydride. The proposed mechanism does not require a direct isomerization of a *trans* to a *cis* structure, a process which ordinarily may require a higher temperature than that of refluxing benzene.

In the course of this work another bisanhydride IX (Figure 5) was obtained. This anhydride, mp 122–124°, was obtained by treatment of methylaconitic acid VIII (Figure 5) with acetyl chloride, and the same bisanhydride presumably, albeit somewhat less pure, mp 117.5–118.5°, was obtained from acid VIII on treatment with acetic anhydride. Both products gave a positive hydroquinone test and both were converted by partial hydrolysis in refluxing benzene to α-methyl-*cis*-aconitic anhydride. It would seem that anhydride IX has the bis(α-methyl-*cis*-aconitic anhydride) anhydride structure albeit the possibility of the bis(γ-methyl-*cis*-aconitic anhydride) anhydride structure or the possibility of a mixed anhydride with one of the anhydride rings not of the maleic anhydride type has not been ruled out. A definitive nmr spectrum of anhydride IX could not be obtained.

Acid VIII, obtained crystalline, mp 179–181°, albeit in poor yield from the crude, was derived according to the reaction sequence of Figure 5. Methyl aconitic acid III was esterified to give the ester mixture VI, the nmr spectrum (Figure 6) indicating two components. Heating ester mixture VI with potassium bisulfate converted one of the components to the other to give the pure ester VII (nmr spectrum of Figure 7) and the hydrolysis of ester VII yielded acid VIII. Comparison of the nmr spectrum (Figure 6) of VI with that (Figure 7) of ester VII shows the disappearance of several lines on going from VI to VII, the peaks at 3.3 (τ 6.7) and 1.96 ppm (τ 8.04) of VI not being present in VII, the spectrum of the ester ethyl groups being simplified, and retention of the

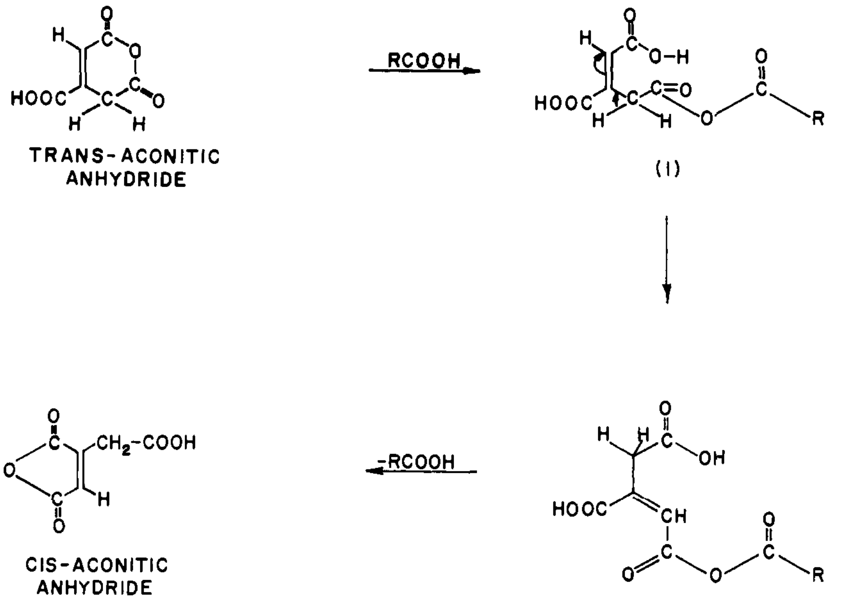


FIGURE 4: A mechanism for thermal isomerization of *trans*-aconitic anhydride to *cis*-aconitic anhydride.

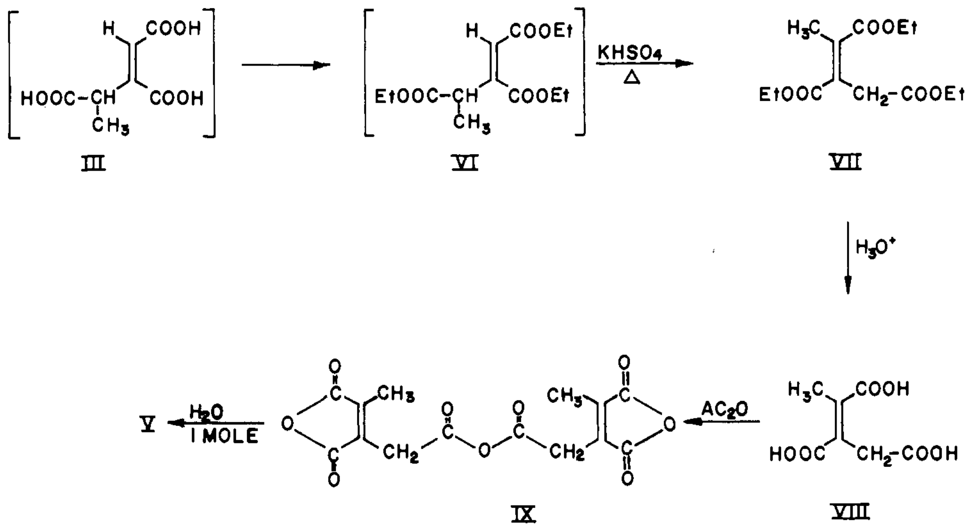


FIGURE 5: Isomers of triethyl α-methylaconitate and a derived methylaconitic acid and anhydride.

peaks at 3.47 (τ 6.53) and 2.10 ppm (τ 7.90). Since the peaks at τ 6.53 and 6.70 are to be ascribed to an uncoupled methylene group and those at τ 7.90 and 8.04 to an uncoupled methyl group, it follows that VI is a mixture of *cis*- and *trans*-α-methylaconitic esters and VII is either *cis*- or *trans*-α-methylaconitic ester. Comparing the nmr spectra of these aconitic esters with the *cis–trans* pairs, dimethyl dimethylmaleate, dimethyl dimethylfumarate and dimethyl citraconate, dimethyl mesaconate, it is noted (Jackman, 1959) that the methyl proton peak is shifted downfield in both pairs on going from the *cis* to the *trans* structure, 0.1 ppm for the dimethylmaleate, dimethyl fumarate pair and 0.2 ppm for the citraconate, mesaconate pair. Accordingly and without consideration of the effect of the γ-carbethoxy group, ester VII is provisionally considered to be triethyl α-methyl-*trans*-aconitate since both the methylene peak and the methyl peak are shifted downfield with respect to the other isomer. While the assignment on this basis is reasonable, it should be pointed out that the τ values for the methylene protons and for the methyl protons, 6.52 and 7.95, respectively, of α-methyl-*cis*-aconitic anhydride are almost identical with those of ester VII. If ester VII is the *trans* ester, then acid VIII, mp 179–180°, would reasonably be α-methyl-*trans*-

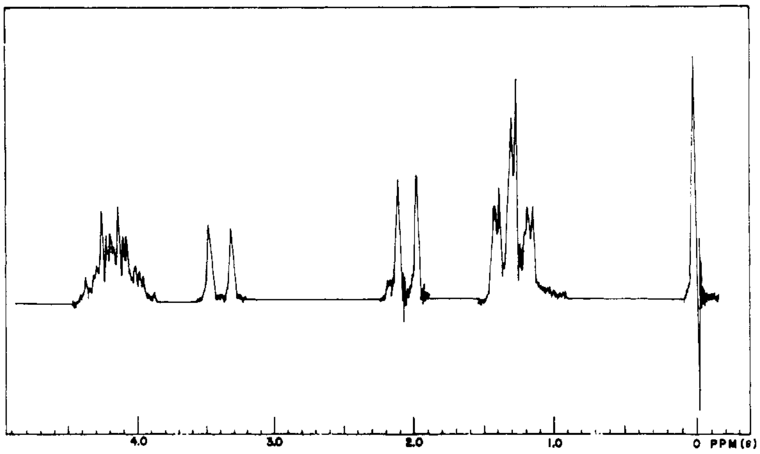


FIGURE 6: Nmr spectrum of the mixed isomers VI of triethyl α-methylaconitate (neat).

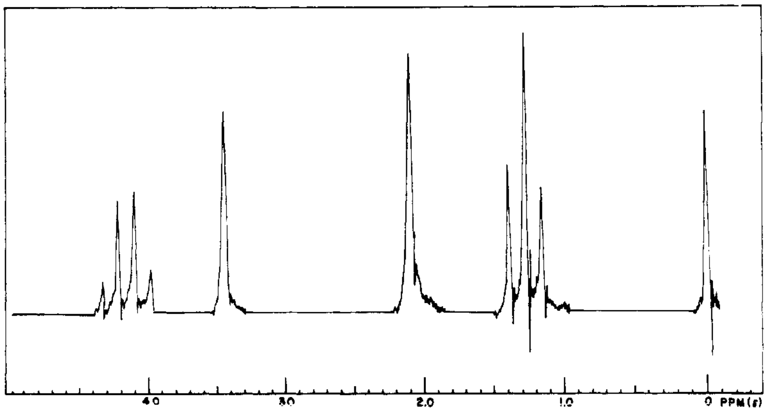


FIGURE 7: Nmr spectrum of the single isomer VII of triethyl α-methylaconitate (neat), presumably triethyl α-methyl-*trans*-aconitate.

aconitic acid and on treatment with acetic anhydride is converted to the *cis*-aconitic anhydride structure as is *trans*-aconitic acid. It is of interest to note that the acid, mp 159°, obtained by Rogerson and Thorpe (1960) and that mp 169–172°, obtained by Anschutz and Deschauer (1906), are, on this basis, either impure or other isomers. It is also of interest to note that Rogerson and Thorpe (1906) obtained from their acid an anhydride, mp 51°, to which was ascribed an itaconic anhydride structure. In view of the similarity of the melting point of this anhydride to that of α-methyl-*cis*-aconitic anhydride, mp 52–53°, Rogerson and Thorpe most likely obtained the latter anhydride.

The stability to the reaction conditions employed of the methyl-*cis*-aconitic anhydride structure *vs.* the *trans*-aconitic anhydride and other possible structures is worthy of comment. Stability of the *cis*-aconitic anhydride structure *vs.* the *trans*-aconitic anhydride structure may be considered without regard to the effect of ring substituents as an example of the Brown–Brewster–Shecter Rule (Brown *et al.*, 1954; Brown, 1957), *i.e.*, six-membered rings with *exo* double bonds being less stable and more susceptible to ring-opening reactions than the corresponding five-membered rings. Stability of the maleic anhydride ring of α-methyl-*cis*-aconitic anhydride and of *cis*-aconitic anhydride *vs.* an itaconic anhydride type of structure is similar to the greater stability of methylmaleic anhydride (citraconic anhydride) compared to itaconic anhydride and is due, presumably, to increase in resonance on conjugation of the double bond with both carbonyl groups of the anhydride ring (Alexander, 1950). Finally, the γ-methyl-*cis*-aconitic anhydride structure is not found, presumably because the anhydride ring is less stable than that of α-methyl-*cis*-aconitic anhydride, an α,α′-disubstituted maleic anhydride.

Anhydride formation from acid III or acid VIII with various ratios of acid to acetic anhydride invariably led to formation of bisanhydride while formation of anhydride from *trans*-aconitic acid invariably gave *cis*-aconitic acid. A ready explanation is not forthcoming, particularly so since the reaction mixtures were not homogeneous and solubility differences may therefore be, at least, in part, responsible (Curtin and Thamm, 1962).

Acknowledgment

We thank Dr. J. W. Hausser for his help in obtaining and interpreting the nmr spectra

References

Alexander, E. R. (1950), Ionic Organic Reactions, New York, N. Y., Wiley, p 283.

Anschutz, R., and Deschauer, A. (1906), *Ann. 347*, 1.

Barker, H. A., Smyth, R. D., Wilson, L. M., and Weissbach, H. J. (1959), *J. Biol. Chem. 234*, 320.

Barker, H. A., Weissbach, H., and Smyth, R. D. (1958), *Proc. Natl. Acad. Sci. U. S. 44*, 1093.

Birch, S. F., Kon, G. A. R., and Norris, W. S. (1923), *J. Chem. Soc. 123*, 1361.

Brown, H. C. (1957), *J. Org. Chem. 23*, 439.

Brown, H. C., Brewster, J. H., and Shecter, H. (1954), *J. Am. Chem. Soc. 76*, 467.

Cox, R. F. B., and McElvain, S. M. (1937), *Org. Syn. 17*, 54.

Cragoe, E. J., Jr., Robb, C. M., and Sprague, J. M. (1950), *J. Org. Chem. 15*, 381.

Curtin, D. Y., and Thamm, R. C. (1962), *J. Org. Chem. 27*, 4674.

Desai, R. D. (1932), *J. Chem. Soc.*, 1091.

Dickman, S. R. (1961), *Enzymes 6*, 495.

Gawron, O., Glaid, A. J., III, Fondy, T. P., and Bechtold, M. M. (1962), *J. Am. Chem. Soc. 84*, 3877.

Gawron, O., and Mahajan, K. P. (1965), *Federation Proc. 24*, 228.

Gawron, O., and Mahajan, K. P. (1966), *Biochemistry 5*, 2343 (this issue; following paper).

Hein, R. W., Astle, M. J., and Shelton, J. R. (1961), *J. Org. Chem. 26*, 4874.

Jackman, L. M. (1959), Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, New York, N. Y., Pergamon, pp 58, 122.

Kessar, S. V., Mahajan, K. P., and Rampal, A. L. (1961), *Current Sci.* (*India*) *30*, 414.

Kessar, S. V., Rampal, A. L., and Mahajan, K. P. (1962), *J. Chem. Soc.*, 4703.

Malachowski, R. (1929), *Ber. 62*, 1323.

Malachowski, R., Giedroyc, M., and Jerzmanowska, Z. (1928), *Ber. 61B*, 2525.

Malachowski, R., and Maslowski, M. (1928), *Ber. 61B*, 2521.

Pfeiffer, P., and Bottler, T. (1918), *Ber. 51B*, 1819.

Rogerson, H., and Thorpe, J. F. (1906), *J. Chem. Soc. 89*, 631.