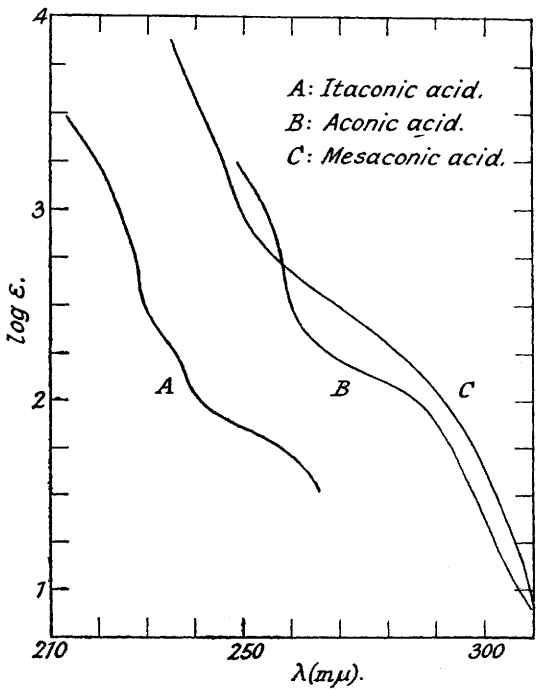
220. *Unsaturated Lactones. Some Esters of Aconic and Coumalic Acids.*

By N. R. Campbell and J. H. Hunt.

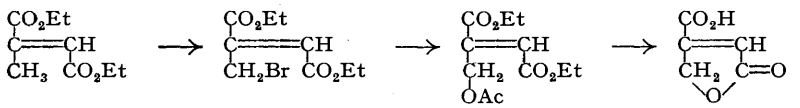
Aconic acid has been synthesised by a new method, which affords positive evidence for its acceptance as a β-substituted αβ-butenolide. The older method of preparation has been developed, and a series of esters of aconic and coumalic acids prepared. The results of simple toxicity tests on the acids and their esters are given.

Among the simpler unsaturated lactones examined by Chen, Elderfield, Steldt, and Fried (*J. Pharmacol.*, 1942, **74**, 381) for cardioactivity, the methyl and ethyl esters of coumalic acid were noteworthy for some positive result. It was considered desirable to extend this series of esters and to compare them biologically with a similar, related, series of esters of aconic acid.



The structure of coumalic acid is well established and probably beyond doubt, but there has been no positive evidence in support of the normally accepted βγ-position of the double bond in aconic acid. The structure of this acid was given by Fittig and Beer (*Annalen*, 1883, **216**, 92) as β-carboxybutenolide, but they were unable to allocate an exact position to the double bond. We have now prepared the αβ-acid by a method which does not involve conditions likely to cause migration, and have identified it with aconic acid prepared by the usual method from itaconic acid.

This new method of preparation involved bromination of the methyl group of diethyl mesaconate (diethyl methylfumarate) by means of *N*-bromosuccinimide, replacement of the bromine by an acetoxy-group under mild conditions, hydrolysis with barium hydroxide, and cyclisation by warming with water.



This evidence of structure brings aconic acid into line with the series of substituted butenolides which includes the aglycones of the digitalis and strophanthus groups of heart-poisons. The latter were also considered to be βγ-butenolides before the work of Elderfield, Paist, Blout, and Uhle (*J. Org. Chem.*, 1941, **6**, 273) who produced evidence suggesting the present accepted αβ-structure.

We have examined the ultra-violet absorption of aconic acid and compared the results with those obtained by Bielecki and Henri (*Ber.*, 1913, **46**, 2602) on mesaconic and itaconic acids, which are open-chain analogues of the two possible structures for aconic acid; we find that the absorption of aconic acid corresponds more closely to that of the fully conjugated isomer, thereby supplying further evidence for the αβ-disposition of the double bond in aconic acid (see Fig.).

The preparation of aconic acid from itaconic acid was also studied. Itaconic acid was prepared by pyrolysis of citric acid (*Org. Synth.*, 1943, Coll. Vol. 2, 368); the isolation of acetone as a by-product together with approximately the theoretical volume of carbon monoxide indicated that acetonedicarboxylic acid was among the primary products of pyrolysis. Itaconic acid was brominated and the resulting " itadibromopyro-tartaric acid " (not isolated) converted into sodium aconate by a modification of the method used by Meilly (*Annalen*, 1874, **171**, 153), giving a greatly improved yield.

Esters of coumalic acid have been prepared by von Pechmann (*Annalen*, 1891, **264**, 279), Ruzicka (*Helv. Chim. Acta*, 1921, **4**, 504), and Caldwell, Tyson, and Lauer (*J. Amer. Chem. Soc.*, 1944, **66**, 1483). The method employed has generally involved treatment of a sulphuric acid solution of coumalic acid with the required alcohol, the coumalic acid being frequently prepared *in situ* from malic acid. We have employed both versions of this method and have also used much smaller (catalytic) quantities of sulphuric acid with azeotropic removal of water. Replacement of sulphuric acid by hydrogen chloride was not successful. We have compared the yields of one ester by the four methods; the first gave a considerably higher yield than the others.

The methyl and ethyl esters of aconic acid have been described by Meilly (*loc. cit.*), Reiter and Bender (*Annalen*, 1905, **339**, 316), and Wislicenus, Böklen, and Reuthe (*ibid.*, 1908, **363**, 353). The most suitable method for preparation of aconic esters is by reaction of silver aconate with the appropriate alkyl halide, usually at elevated temperature. With the exception of the *n*-butyl ester, the aconates prepared have all been obtained as crystalline solids; the *iso*propyl ester has been obtained in liquid and solid forms, both with satisfactory analyses. Ethyl aconate was described by Wislicenus (*loc. cit.*) as a liquid; under identical conditions we obtained a crystalline product.

While Rast's method gave satisfactory results for the molecular weights of all coumalates prepared, the aconates showed values ranging from theoretical to three times theoretical. We have been unable to explain this, or to correlate these anomalous results with the existence of solid and liquid forms.

Biological Investigation.—As a result of solubility difficulties in testing of these and other compounds by the usual assay method for digitalis, we adopted a simple toxicity test, the assumption being made that high cardioactivity might be accompanied by high toxicity. The substances were dissolved or suspended in arachis oil and administered subcutaneously to mice; median lethal doses (L.D./50) were calculated from the number of deaths after four days.

Toxicities in the coumalate series appear to decrease with increasing length of the alkyl chain, rather than with increasing molecular weight; the level of toxicity in the aconate series was so low that massive doses would be required for any variations to become apparent. It seems improbable that notable toxicity or cardioactivity will be found among simple esters of either of these acids.

	L.D./50.		L.D./50.
Coumalic acid	0·8 mg./g.	n-*Butyl coumalate*	>1 mg./g.
Methyl coumalate	0·3 mg./g.	iso*Butyl coumalate*	0·6 mg./g.
Ethyl coumalate	0·33 mg.g/.	*Benzyl coumalate*	>1 mg./g.
n-*Propyl coumalate*	0·55 mg./g.	cyclo*Hexyl coumalate*	0·55 mg./g.
iso*Propyl coumalate*	0·4 mg./g.		

Aconic acid, methyl aconate, ethyl aconate, n-*propyl aconate*, iso*propyl aconate*, n-*butyl aconate*, iso*butyl aconate*, *benzyl aconate*, and cyclo*hexyl aconate*, all showed L.D./50 >1 mg./g.

Experimental.

Sodium Aconate.—Itaconic acid (260 g.) was stirred to a paste with water (340 c.c.), and bromine (320 g.) slowly added, the temperature not being allowed to rise above 50°. When all but a trace of bromine had disappeared, the solution was neutralised with sodium hydrogen carbonate (336 g.). The mixture was then heated to 50° on the water-bath and treated with a suspension of sodium carbonate (106 g., anhydrous) in water (158 c.c.) at 50°, added in small portions until the solution remained neutral to bromothymol-blue. The mixture was cooled and allowed to remain at 0° for 1 hour. The crystalline sodium salt was filtered off, washed with iced water and with 95% alcohol, and dried in a vacuum at 110°. Yield of anhydrous sodium aconate, 179 g. (59%).

Silver Aconate.—Sodium aconate (100 g.) was stirred with water (80 c.c.) and heated to 40°. Silver nitrate (112·5 g.) dissolved in warm water (40 c.c.) was added, the mixture cooled to 0°, and the crystalline precipitate filtered off, washed with iced water and methanol, and dried in a vacuum desiccator. Yield, 147 g. (94%).

Aconic Acid.—Anhydrous sodium aconate (10 g.) was suspended in dry ether (30 c.c.). Dry hydrogen chloride was then passed in with stirring, until a gain in weight of 3·0 g. was obtained. The mixture was left overnight, the solid (11·5 g.) filtered off and extracted with ether in a continuous extractor. Removal of solvent left 7·6 g. of aconic acid, m.p. 162°. A further 0·7 g. of impure aconic acid, m. p. 156°, was obtained by evaporation of the original mother liquor. Total yield. 97%.

Ethyl Aconate (cf. Wislicenus, *loc. cit.*).—Silver aconate (30 g.) and ethyl iodide (15 c.c.) were heated in a sealed tube at 100° for 4 hours. The contents of the tube were extracted with ether, the extract washed with sodium hydrogen carbonate solution and water and dried ($MgSO_4$), and the solvent removed. The residue (15·5 g.) was distilled at reduced pressure, 8·95 g. of partly crystalline material (b. p. 132°/15 mm.) being collected. The crystals were separated and recrystallised from methanol at − 10°. Yield, 3·3 g.; m. p. 64° (Found: C, 53·87; H, 5·31; *M*, 478. Calc. for $C_7H_8O_4$: C, 53·51; H, 5·13%; *M*, 157).

n-*Propyl Aconate.*—Silver aconate (20 g.) was refluxed for 1½ hours with *n*-propyl bromide (20 c.c.), excess of bromide then distilled off, and the residue diluted with ether and filtered. The brown oil (10·1 g.) remaining after removal of the ether was distilled, yielding 4·4 g. of partly crystalline material (b. p. 80—88°/0·1 mm.) which was dried on a porous tile. The *ester*, recrystallised from benzene–*cyclo*hexane, had m. p. 34° (Found: C, 56·2; H, 6·04; *M*, 286. $C_8H_{10}O_4$ requires C, 56·5; H, 5·92%; *M*, 170).

iso*Propyl Aconate.*—(*a*) Silver aconate (14 g.) and *iso*propyl bromide (10 c.c.) were heated in a sealed tube at 100° for 3 hours. The contents of the tube were extracted with ether and the ether and excess of *iso*propyl bromide removed by distillation, leaving a brown oil (5 g.). Distillation of this yielded the *ester* as a colourless oil (1·7 g.), b. p. 70—71°/0·1 mm., $n_D^{20°}$ 1·460 (Found: C, 56·4; H, 5·58; *M*, 213. $C_8H_{10}O_4$ requires C, 56·5; H, 5·92%; *M*, 170).

(*b*) *iso*Propyl iodide (14 c.c.) was added slowly to silver aconate (28 g.) and benzene (30 c.c.). On gently heating the mixture a violent reaction commenced, necessitating cooling. The mixture was then refluxed for 1½ hours and cooled. The insoluble material was filtered off, washed with benzene, and extracted with ether in a continuous extractor. The ethereal extract yielded aconic acid (4·0 g.). The benzene solution was washed with sodium hydrogen carbonate solution, then with water, and dried ($MgSO_4$). Removal of the benzene gave a brown oil (9·48 g.), which on distillation gave two fractions: (i) b. p. 88—92°/0·2 mm., (ii) b. p. 100—120°/0·1 mm. Fraction (ii) which was partly crystalline was dried on a porous tile and recrystallised from light petroleum (b. p. 40—60°); m. p. 80° (Found: C, 56·3; H, 5·81%; *M*, 172).

n-*Butyl Aconate.*—Silver aconate (12·7 g.) was heated with *n*-butyl iodide (8 c.c.) and benzene (20 c.c.) in a sealed tube at 100° for 1 hour. The product, treated as for *n*-propyl aconate, yielded the *ester* as a colourless oil (2·5 g.), b. p. 91°/0·1 mm., $n_D^{20°}$ 1·471 (Found: C, 58·8; H, 6·66; *M*, 491. $C_9H_{12}O_4$ requires C, 58·7; H, 6·57%; *M*, 184).

iso*Butyl Aconate.*—Silver aconate (25·4 g.) was refluxed for 1½ hours with *iso*butyl bromide (14 c.c.) and benzene (30 c.c.). The mixture was diluted with ether, filtered, and the filtrate washed with sodium hydrogen carbonate solution, then with water, and dried ($MgSO_4$). After removal of the ether the crude oily product (9·8 g.) was distilled under reduced pressure. The fraction, b. p. 86—88°/0·1 mm., partly crystallised and was dried on a porous plate. The solid *ester* was recrystallised from light petroleum (b. p. 60—80°); m. p. 114° (Found: C, 58·7; H, 6·58; *M*, 200. $C_9H_{12}O_4$ requires C, 58·7; H, 6·57%; *M*, 184).

Benzyl Aconate.—Silver aconate (11·5 g.) was refluxed for 1 hour with benzene (20 c.c.) and benzyl chloride (8·4 c.c.). The filtered benzene solution was washed with sodium hydrogen carbonate solution, then with water, and dried ($MgSO_4$). After removal of the benzene the *ester* was distilled under reduced pressure, b. p. 140°/0·04 mm., and recrystallised from *cyclo*hexane. Yield, 1·1 g.; m. p. 59—60° (Found: C, 66·03; H, 4·55; *M*, 384. $C_{12}H_{10}O_4$ requires C, 66·04; H, 4·62%; *M*, 218).

cyclo*Hexyl Aconate.*—Aconic acid (6·25 g.) was dissolved in cold concentrated sulphuric acid (10 c.c.), the solution cooled to 0°, and *cyclo*hexanol (15·8 c.c.) added. The mixture was allowed to warm to 18°, left for 20 hours, then poured on crushed ice and extracted with ether. After being washed, dried, and freed from solvent, the *ester* was crystallised from pentane (3·4 g.) and recrystallised from light petroleum (b. p. 60—80°); m. p. 68° (Found: C, 62·88; H, 6·72; *M*, 295. $C_{11}H_{14}O_4$ requires C, 62·84; H, 6·71%; *M*, 210).

iso*Propyl Coumalate.*—Malic acid (50 g.) was added slowly with stirring to fuming sulphuric acid (150 g.) containing 10% of sulphur trioxide, and the mixture heated on the water-bath until evolution of gas ceased. The mixture was then cooled and *iso*propyl alcohol (165 c.c.) added. After being heated on the water-bath for 1 hour under reflux, the mixture was cooled, poured on ice (200 g.), partly neutralised with sodium carbonate, left overnight, and filtered. The filtrate was extracted with ether, and the extract washed, dried and freed from solvent. The residual oil was distilled under reduced pressure. The fraction, b. p. 102—110°/0·1 mm., deposited crystals of the *ester* which were twice crystallised from light petroleum (b. p. 40—60°). Yield, 6 g.; m. p. 44° (Found: C, 59·4; H, 5·35; *M*, 171. $C_9H_{10}O_4$ requires C, 59·3; H, 5·41%; *M*, 182).

n-*Butyl Coumalate.*—Malic acid (100 g.), fuming sulphuric acid (300 g.), and *n*-butyl alcohol (200 c.c.) were brought into reaction by the method employed for *iso*propyl coumalate. After distillation of the crude reaction mixture, the fraction, b. p. 120—124°/0·1 mm., solidified on standing. The *ester* was twice recrystallised from light petroleum (b. p. 40—60°). Yield, 10 g.; m. p. 41° (Found: C, 61·4; H, 6·14; *M*, 191. $C_{10}H_{12}O_4$ requires C, 61·2; H, 6·16%; *M*, 196).

n-*Propyl Coumalate.*—(*a*) Malic acid (100 g.), fuming sulphuric acid (300 g.), and *n*-propyl alcohol (176 g.) were brought into reaction by the method employed for *iso*propyl coumalate. Yield of pure ester, 14 g. (20%).

(*b*) Coumalic acid (6·8 g.) was dissolved in concentrated sulphuric acid (13 g.) and cooled to room temperature. *n*-Propyl alcohol (13 g.) was added, and the mixture heated with occasional shaking, on the water-bath, under reflux for 1 hour and poured into iced water (50 c.c.). The solid was filtered off, washed, and dried (6 g.). The filtrate was extracted with ether and a further quantity of solid obtained, which was added to that previously obtained and distilled under reduced pressure. The fraction, b. p. 96—100°/0·1 mm., was crystallised from light petroleum (b. p. 60—80°). Yield of *ester*, 5 g. (57%); m. p. 59—60° (Found: C, 59·8; H, 5·74; *M*, 188. $C_9H_{10}O_4$ requires C, 59·3; H, 5·41%; *M*, 182).

(*c*) Coumalic acid (6·8 g.) was covered with *n*-propyl alcohol (13 g.) and saturated with dry hydrogen chloride at 0°. The mixture was allowed to warm to room temperature, and next day was poured into water. No ester could be isolated; unchanged coumalic acid (5 g.) was recovered.

(*d*) A mixture of coumalic acid (6·8 g.), *n*-propyl alcohol (10·6 c.c.), toluene (10 c.c.), and concentrated sulphuric acid (2 drops) was distilled slowly, the distillate being dried (K_2CO_3) and returned. After 3 hours toluene and excess of propyl alcohol were distilled off. Yield of ester, 3 g. (34%).

iso*Butyl Coumalate.*—Coumalic acid (7·1 g.), concentrated sulphuric acid (15 g.), and *iso*butyl alcohol (17·5 c.c.) were brought into reaction by the method employed for *n*-propyl coumalate. The crude *ester*

was distilled and the fraction, b. p. 120—126°/0·1 mm., twice crystallised from light petroleum (b. p. 40—60°). Yield, 6 g.; m. p. 62° (Found: C, 61·1; H, 6·21; *M*, 199. $C_{10}H_{12}O_4$ requires C, 61·2: H, 6·12%; *M*, 196).

Benzyl Coumalate.—Silver coumalate (4·5 g.) (von Pechmann, *loc. cit.*) was refluxed for 1 hour with benzyl chloride (5 c.c.) and benzene (15 c.c.). The filtered benzene solution was washed and dried. Removal of the benzene left an oil (2·7 g.), which was distilled under reduced pressure, b. p. 140—150°/0·05 mm., and crystallised twice from ether. Yield of *ester*, 0·52 g.; m. p. 92° (Found: C, 67·9; H, 4·19; *M*, 238. $C_{13}H_{11}O_4$ requires C, 67·8; H, 4·35%; *M*, 230).

cyclo*Hexyl Coumalate.*—Coumalic acid (12·6 g.) was dissolved in concentrated sulphuric acid (25 c.c.) and the mixture cooled to 0°. *cyclo*Hexanol (45 c.c.) was added and the mixture allowed to warm to room temperature and left for 3 days. The mixture was worked up as under *cyclo*hexyl aconate, yielding 6·5 g. of crude crystalline *ester* which after further crystallisation (charcoal) from light petroleum (b. p. 60—80°) had m. p. 70° (Found: C, 65·09; H, 6·46; *M*, 224. $C_{12}H_{14}O_4$ requires C, 64·8; H, 6·35%; *M*, 222).

Diethyl γ-Bromomesaconate.—(*a*) Diethyl mesaconate (71 g.) was refluxed with *N*-bromosuccinimide (71 g.) and carbon tetrachloride (213 c.c.) for 30 hours. The mixture was cooled, filtered, freed from solvent, and diluted with light petroleum (b. p. 40—60°) (200 c.c.) which precipitated traces of succinimide. After filtration and removal of solvent the *ester* was distilled, b. p. 91°/0·1 mm. Yield, 52 g. (Found: C, 39·8; H, 5·07; Br, 33·6. $C_9H_{13}O_4Br$ requires C, 40·77; H, 4·94; Br, 30·14%).

(*b*) Diethyl mesaconate (48·5 g.) was heated under reflux with *N*-bromosuccinimide (31 g.), carbon tetrachloride (50 c.c.), and benzoyl peroxide (2·4 g.) for 1 hour. The mixture was filtered and the filtrate washed and dried. After removal of solvent the residue (60·0 g.) was distilled; unchanged diethyl mesaconate (18·2 g., b. p. 60—65°/0·15 mm.) was followed by the main fraction, b. p. 94—98°/0·15 mm. (31·3 g.), which was redistilled; b. p. 72°/0·10 mm., $n_D^{20°}$ 1·485 (Found: C, 40·71; H, 5·04; Br, 31·3%). Repeated distillation of the ester failed to give a product with a correct bromine analysis.

Aconic Acid.—Diethyl γ-bromomesaconate (10 g.) was heated for 1 hour on the water-bath, under reflux, with potassium acetate (5 g. fused) in absolute alcohol (50 c.c.). The mixture was cooled and filtered, most of the alcohol distilled off, and the residue taken up in benzene. The resulting oil was distilled, 7·23 g. being collected, b. p. 96°/0·1 mm.

The crude acetoxy-derivative (4·2 g.) was stirred for 1½ hours at room temperature with water (50 c.c.), and barium hydroxide (8·15 g.) was gradually added. The slightly cloudy solution was filtered and treated with 5N-sulphuric acid (10·5 c.c.). After removal of the barium sulphate, the solution was heated at 70° for ½ hour and then gave a deep purple colour in the Legal test whereas no colour was produced before heating. Extraction with ether in a continuous extractor gave a mass of sticky crystals which were washed with a little dry ether. Yield, 0·6 g. of crystals, m. p. 164°, which did not depress the m. p. of aconic acid (Found: C, 46·84; H, 3·31. Calc. for $C_5H_4O_4$: C, 46·89; H, 3·14%). The methyl ester (from the silver salt and methyl iodide) had m. p. 82° and did not depress the m. p. of methyl aconate.

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