generally within ten to twelve hours. The catalyst was filtered and the solution evaporated to dryness under reduced pressure. With the exception of the diethylamino derivative, all the dihydrobenzofuran amino alcohol hydrochlorides appeared as colorless solids, and were recrystallized from absolute alcohol–ether.

Debenzylation Experiments.—Hydrogenolysis of the hydrochlorides of 2-dibenzylaminoacetylbenzofuran [II, $-N(CH_2C_6H_5)_2$] to 2-benzylaminoacetylbenzofuran (II, $-NHCH_2C_6H_5$), of 2-benzylmethylaminoacetylbenzofuran [II, $-N(CH_3)CH_2C_6H_5$] to 2-methylaminoacetylbenzofuran (II, $-NHCH_3$), of 1-(2-benzofuryl)-2-dibenzylamino-ethanol [III, $-N(CH_2C_6H_5)_2$] to 1-(2-benzofuryl)-2-benzylaminoethanol (III, $-NHCH_2C_6H_5$), and of 1-(2-benzofuryl)-2-benzylmethylaminoethanol [III, $-N(CH_3)CH_2C_6H_5$] to 1-(2-benzofuryl)-2-methylaminoethanol (III, $-NHCH_3$) was carried out with prereduced palladized charcoal under the same conditions as those described above for the preparation of the 2,3-dihydrobenzofuran amino alcohols (IV). The time required for the debenzylation of 1 g. samples varied from two to thirty minutes.

2-(1-Ethyl-1-hydroxy-2-piperidino)-benzofuran.—A solution of 8.5 g. of 2-piperidinoacetylbenzofuran in 150 cc. of dry benzene was added gradually to a stirred ice-cold solution of ethylmagnesium iodide prepared from 1.7 g. of magnesium and 11.6 g. of ethyl iodide in 20 cc. of dry ether. After completion of the addition, the ether was distilled off, the mixture refluxed for two hours, and hydrolyzed with 20 g. of ice and 45 cc. of a cold 25% ammonium chloride solution. The tertiary alcohol was extracted into benzene, the extract dried over sodium sulfate, the solvent distilled under reduced pressure, and the oily residue converted to the hydrochloride.

2-(3-Piperidinopropionyl)-benzofuran.—A mixture of 25.5 g. of 2-acetylbenzofuran, 25 g. of piperidine hydrochloride, 6.7 g. of paraformaldehyde, 50 cc. of isoamyl alcohol, and four drops of ethanolic hydrogen chloride was refluxed for two minutes when the amino ketone hydrochloride precipitated. After cooling, it was filtered and recrystallized from ethanol.

Reduction of the β-piperidino ketone hydrochloride with aluminum isopropoxide was carried out as described above for the series of α-amino ketones. Likewise, hydrogenation of the β-piperidino alcohol with palladized charcoal followed the pattern set for the lower homologs.

1,4-Dibenzyl-1,4-dihydro-2,5-di-(2-benzofuryl)-pyrazine.—A solution of 3 g. of 2-bromoacetylbenzofuran in 35 cc. of dry benzene was added slowly to a solution of 2 g. of benzylamine in 15 cc. of benzene. After standing for ten hours at room temperature, the precipitated benzylammonium bromide was filtered, the solvent removed from the filtrate *in vacuo*, and the residue heated at 100° and 1 mm. for thirty minutes. It was dissolved in ether, the solution filtered from much tar, and neutralized with ethereal hydrogen chloride. The tan dihydrochloride, crystallized from ethanol–ether, melted at 220°. The yield was 0.5 g.

Anal. Calcd. for $C_{44}H_{36}N_2O_2 \cdot 2HCl$: Cl, 12.5. Found: Cl, 12.1.

2-Cyanoacetylbenzofuran.—A suspension of 12 g. of 2-bromoacetylbenzofuran in 60 cc. of ethanol was added to a solution of 9 g. of sodium cyanide in 30 cc. of water. The temperature rose to 50°. After stirring at 50–55° for one-half hour, the mixture was cooled, diluted with 100 cc. of water, filtered with the aid of Darco, and the filtrate acidified with concentrated hydrochloric acid. The cyano ketone separated out. The yellow crystals were filtered, washed, and recrystallized from acetone.

One-half gram of the cyano ketone was cleaved by boiling with 25 cc. of a 3% potassium hydroxide solution for two hours until no more ammonia was evolved. The solution was cooled, acidified, and the white precipitate of benzofuran-2-carboxylic acid filtered, washed and dried. It melted at 191°, and a mixture melting point with a sample of coumarilic acid showed no depression.

Color Reactions.—The dialkylamino ketones and alcohols described in this article exhibited distinctive color reactions with cold concentrated sulfuric acid. The amino ketones II and VIII gave a deep yellow, the amino alcohols III a deep blue, the amino alcohols IX a deep green, and the coumaran derivatives IV and X a deep red color. The corresponding dibenzylamino compounds showed the same color reactions but in a much less marked degree.

Summary

The synthesis of a number of 2,3-dihydrobenzofuryl amino alcohols containing secondary and tertiary alcohol and amino groups has been described. Certain side-reactions encountered in the course of the syntheses have been discussed.

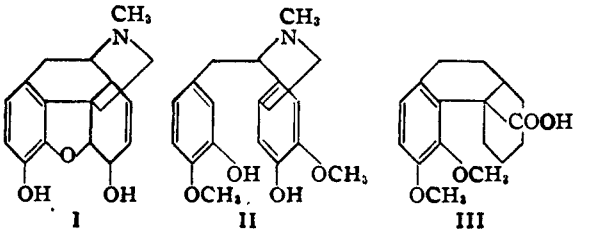
CHARLOTTESVILLE, VA. RECEIVED OCTOBER 23, 1944

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

Experiments on the Synthesis of Compounds Related to Morphine. I. The Internal Michael Reaction

BY C. F. KOELSCH

Although twenty years have elapsed since the proposal of an adequate structure for morphine (I)[1] little progress has been made toward the synthesis of this important alkaloid. A projected synthesis of a portion of the morphine molecule by Manske[2] was abandoned in its early stages. Researches by Robinson and co-workers[3] were directed toward morphine through bases of the laudanosine type, but the hoped for "fortunate and partly fortuitous discovery" of a way to transform these substances (*e. g.*, "protosinomenine," II) into phenanthrene alkaloids has not yet been made. Experiments by Schöpf and co-

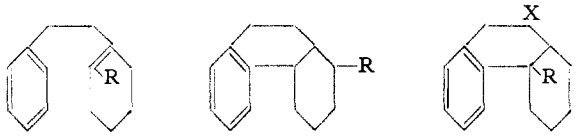


(1) Gulland and Robinson, *Mem. Manchester Phil. Soc.*, **69**, 79 1925).

(2) Manske, THIS JOURNAL, **53**, 1104 (1931).

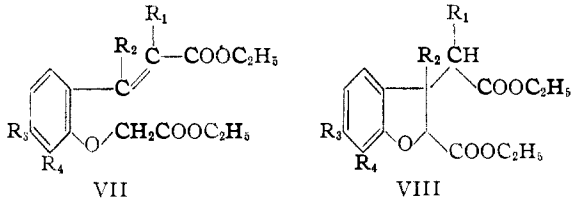
(3) Robinson and Sugasawa, *J. Chem. Soc.*, 3163, 3173 (1931); 789 (1932): 280 (1933); Kitasato and Robinson, *ibid.*, 785 (1932).

workers[4] carried out along the same lines as those of Robinson, also had a negative outcome. Reactions investigated by Fieser and Holmes[5] and by Holmes and Trevoy[6] led to III and related substances in which C-13 is quaternary, but it has been indicated that the group attached to C-13 cannot be converted into an aminoethyl chain. An alternative approach through 4-alkyl-1,2-naphthoquinones likewise appears unfeasible.[7] The most notable synthetic advances have been made by Grewe[8] who has shown that although compounds of type IV undergo cyclization to V when R is CH_2COOH or $CH_2CH_2N(CH_3)_2$, they yield VI when R is CH_3 or $CH_2CH{=}CH_2$. Compound VI (R = C_3H_5) was converted into VI (R = CH_2CHO), but attempts to obtain a molecule (VI, R = C_3H_5) with X = COOH or NH_2 were not entirely successful.

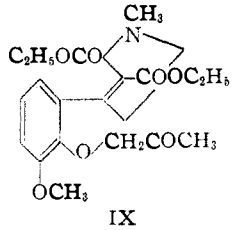


IV V VI

In the present paper it is reported that compounds of type VII will undergo cyclization through an internal Michael reaction. This cyclization is apparently not subject to an inhibiting effect by substituents on the α- and β-carbon atoms, as are intermolecular Michael reactions. The yields obtained indicate that the reaction is essentially complete, again in contrast to intermolecular Michael reactions, where unfavorable equilibria must often be reckoned with.



VII VIII



IX

The reaction opens many routes to the synthesis of compounds related to morphine, for example, a noteworthy intermediate would be compound IX. Research is being carried out in this Laboratory directed to the preparation of IX and to the exploration of several additional routes to morphine made possible by the new reaction and in other ways.

Ethyl 2-Carbethoxycoumaran-3-acetate (VIII, $R_1 = R_2 = R_3 = R_4 = H$)

A solution of 12 g. of sodium in 160 ml. of dry ethanol was treated with 73 g. of coumarin and boiled for ten minutes. Sixty-four grams of ethyl chloroacetate was then added, and boiling was continued for three hours. The mixture was then poured into one liter of ice-water, and the precipitated substance was pressed out on a filter and washed with dilute alcohol. Crystallization from dilute alcohol gave 81.5 g. (58%) of **ethyl coumarate-O-acetate** (VII, $R_1 = R_2 = R_3 = R_4 = H$)[9] colorless prisms, m. p.[10] 56–58°.

Anal.[11] Calcd. for $C_{15}H_{18}O_5$: C, 64.8; H, 6.5. Found[R]: C, 65.0; H, 6.4.

From the aqueous alcoholic mother liquors, there was obtained 45 g. of oil, which was found to contain 4 g. of ethyl ethoxyacetate, 9.5 g. of coumarin, and 28.5 g. of a mixture, b. p. 195–225° at 10 mm., of ethyl coumarate-O-acetate and ethyl coumarinate-O-acetate (see below).

Coumaric Acid-O-acetic Acid,[12] obtained by saponifying the ester with 10% aqueous potassium hydroxide, formed colorless prisms from acetic acid, m. p. 222–224°.

Anal. Calcd. for $C_{11}H_{10}O_5$: C, 59.5; H, 4.5; N. E., 111. Found[K]: C, 59.6; H, 4.9; N. E., 111.5.

The barium **salt** formed difficultly soluble matted white needles. For analysis it was dried at room temperature over sulfuric acid.

Anal. Calcd. for $C_{11}H_8O_5Ba + 3H_2O$: H_2O, 13.1; Ba, 33.4. Found[K]: H_2O, 13.4; Ba, 33.2.

A warm solution of 0.6 g. of the acid in 5 ml. of dilute potassium carbonate decolorized 1.4 g. of potassium permanganate (calcd. 1.42 g.); acidification gave salicylic acid-O-acetic acid, m. p. and mixed m. p. 191–192° (reported,[13] 191.5–192°).

A solution of 2.2 g. of the acid in 25 ml. of water containing 1 g. of sodium hydroxide and 0.5 g. of Raney nickel was shaken with hydrogen at 25° and 750 mm.[14] The product, **melilotic acid-O-acetic acid**, separated from water in the form of stout colorless prisms (2.1 g.), m. p. 141–142°.

Anal. Calcd. for $C_{11}H_{12}O_5$: C, 58.9; H, 5.4. Found[R]: C, 59.1; H, 5.4.

When a solution of 0.25 g. of sodium in 4 ml. of dry ethanol was added to 14 g. of ethyl coumarate-O-acetate at 25°, the mixture became deep yellow and its temperature rose to 75°. It was warmed on a water-bath for fifteen minutes, then neutralized with acetic acid and treated with ether and dilute sodium carbonate. The neutral product was distilled, giving 10.7 g. (77%) of **ethyl 2-carbethoxycoumaran-3-acetate**, b. p. 191–193° at 9 mm.

(4) Schöpf and Thierfelder, *Ann.*, **497**, 22 (1932); **537**, 143 (1939); Schöpf, Perrey and Jäckh, *ibid.*, **497**, 47 and 59 (1932).

(5) Fieser and Holmes, THIS JOURNAL, **58**, 2319 (1936); **60**, 2548 (1938).

(6) Holmes and Trevoy, *Can. J. Research*, **B22**, 56, 109 (1944).

(7) Fieser and Bradsher, THIS JOURNAL, **61**, 417 (1939).

(8) Grewe, *Ber.*, **72**, 426, 785, 1314 (1939); **76**, 1072, 1076 (1943).

(9) The same ester was also obtained from recrystallized ethyl coumarate [prepared by the method of Fries and Klostermann, *Ann.*, **362**, 11 (1908)], alcoholic sodium ethoxide, and ethyl chloroacetate. This preparation confirms the configuration assigned to the ester.

(10) All m. p.'s in the present paper were observed using a calibrated thermometer.

(11) Analyses marked [M] by Perry Morgan, [R] by S. T. Rolfson, [K] by the author.

(12) This structure has been ascribed by Rössing [*Ber.*, **17**, 2988 (1884)] to an acid m. p. 190°, obtained from *o*-formylphenoxyacetic acid, acetic anhydride, and sodium acetate. It is not possible to reconcile Rössing's description with the present data; if his acid were a mixture of *cis*- and *trans*-forms, it should have yielded a difficultly soluble barium salt, and reduction should have converted it into the easily crystallizable melilotic acid-O-acetic acid. Attempts in this Laboratory to repeat Rössing's preparation gave only coumarone, a product also obtained by Rössing; *cf.* Dumont and v. Kostanecki, *Ber.*, **42**, 912 (footnote 3) and 913 (1909).

(13) v. Auwers and Haymann, *Ber.*, **27**, 2802 (1894).

(14) Figure 1 shows the interesting effect of substituents on this type of reduction.

Anal. Calcd. for $C_{13}H_{18}O_5$: C, 64.8; H, 6.5. Found[R]: C, 64.6; H, 6.8.

2-Carboxycoumaran-3-acetic acid, obtained by saponifying the ester with 10% aqueous sodium hydroxide, separated from water in the form of hydrated needles that melted below 100°; from ethyl acetate–ligroin it crystallized in the form of needles, m. p. 170–172°. Its solution in sodium carbonate was stable to permanganate.

Anal. Calcd. for $C_{11}H_{10}O_5$: C, 59.5; H, 4.5; N. E., 111. Found[R]: C, 59.6; H, 5.0; N. E., 110.

The **silver salt** formed white micro-crystals, difficultly soluble in water.

Anal. Calcd. for $C_{11}H_8O_5Ag_2$: Ag, 49.6. Found[K]: Ag, 49.9.

Because of the data reported by Rössing,[12] it was necessary to prepare coumarinic acid-O-acetic acid; this substance was found to be different from Rössing's acid.

Coumarin (14.6 g.) was boiled for fifteen minutes with 10 g. of sodium hydroxide in 40 ml. of water and 20 ml. of alcohol. The resulting yellow solution was then mixed with a solution of 10 g. of chloroacetic acid and 5.6 g. of sodium carbonate in 20 ml. of water and boiled for thirty minutes more. The solution was acidified, boiled for a few minutes, and then made slightly basic with sodium carbonate. The undissolved coumarin (9.4 g.) was removed, and the mother liquor was acidified. There was obtained 3.5 g. of **coumarinic acid-O-acetic** acid, colorless needles from ethyl acetate–ligroin, m. p. 149–151°; the compound was quite soluble in water.

Anal. Calcd. for $C_{11}H_{10}O_5$: C, 59.5; H, 4.5; N. E., 111. Found[K]: C, 59.6; H, 4.8; N. E., 111.5.

The **silver salt** was difficultly soluble in water.

Anal. Calcd. for $C_{11}H_8O_5Ag_2$: Ag, 49.6. Found[K]: Ag, 50.0.

The **barium salt** formed a fine white powder, nearly insoluble in hot or cold water. Unlike the barium salt of the stereoisomeric acid, it contained no water after it had been dried at room temperature over sulfuric acid.

Anal. Calcd. for $C_{11}H_8O_5Ba$: Ba, 38.5. Found[K]: Ba, 38.7.

The *cis*-acid was rapidly reduced to melilotic acid-O-acetic acid, m. p. and mixed m. p. 141–142°, when its solution in dilute sodium hydroxide was shaken with hydrogen in the presence of Raney nickel.

2-Carboxycoumaran-3-acetic acid was alternatively synthesized through *o*-formylphenoxyacetic acid in the following way.

Ethyl *o*-formylphenoxyacetate[15] (57 g.) was shaken with a warm solution of 12 g. of sodium hydroxide in 100 ml. of water until solution had taken place (5 minutes). The *p*H was adjusted to about 11, and 110 ml. of 2.65 molar sodium cyanoacetate[16] was added at 40°. The mixture became warm (55°) and soon solidified. After one hour, it was acidified to congo red, giving 53.8 g. of nearly pure **α-cyanocoumaric acid-O-acetic acid**; from dilute acetic acid, the compound separated as a pale yellow powder that sintered at 220° and melted with gas evolution at about 240°.

Anal. Calcd. for $C_{12}H_9NO_5$: C, 58.2; H, 3.6. Found[K]: C, 58.5; H, 3.9.

When 54 g. of the cyano dibasic acid was heated in quinoline, carbon dioxide was evolved at 140°. The temperature was raised during five minutes to 240°, and most of the quinoline was removed by distillation at 20 mm. The residue was dissolved in 150 ml. of 10% sodium carbonate, washed thoroughly with ether, and then acidified. There was obtained 42 g. (95%) of a mixture of *cis*- and *trans*-monobasic acids that could not be separated into its constituents by fractional crystallization.

Five and one-half grams of this mixture was boiled for fifteen minutes with 30 ml. of 1% alcoholic hydrogen chloride, and the excess alcohol was then removed under reduced pressure. The residue was taken up in ether and washed with dilute sodium carbonate, giving 5.3 g. of a mixture of *cis*- and *trans*-cyano esters. Distillation at 18 mm. followed by fractional crystallization from alcohol gave about 3.5 g. of **ethyl coumaronitrile-O-acetate**, b. p. 225–227° at 18 mm., coarse colorless plates from alcohol, m. p. 91–92°, and about 1 g. of **ethyl coumarinonitrile-O-acetate**, b. p. 215–218° at 18 mm., colorless prisms from benzene–ligroin, m. p. 69–71°.

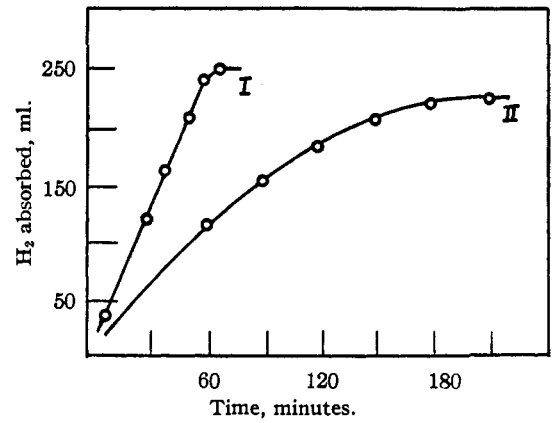


Fig. 1.—Absorption of hydrogen (740 mm., 25°) by 0.01 mole of the sodium salts of coumaric acid-O-acetic acid (Curve I), and β,4-dimethylcoumarinic acid-O-acetic acid (Curve II). Curves III and IV, representing the behaviors of α-ethyl-β,4-dimethyl- and 4-methyl-α,β-trimethylenecoumarinic acids, coincide with the time axis, and are not detailed in the figure.

Anal. Calcd. for $C_{13}H_{13}NO_3$: C, 67.5; H, 5.6. Found: (*trans*)[K] C, 67.2; H, 5.5; (*cis*)[M] C, 67.5; H, 5.5.

Complete saponification of the separated nitrile-esters gave the corresponding dibasic acids, identical with the compounds obtained from coumarin. Partial saponification of the separated esters (0.5 g.) with 0.1 g. of sodium hydroxide in 0.2 ml. of water and 3 ml. of alcohol for thirty seconds at 60° gave the respective nitrile-acids. **Coumaronitrile-O-acetic acid** formed faintly yellow needles from 20% acetic acid, m. p. 137–139°. **Coumarinonitrile-O-acetic acid** formed colorless flat needles from benzene, or colorless prisms from dilute acetic acid, m. p. 160–162°.

Anal. Calcd. for $C_{11}H_9NO_3$: C, 65.0; H, 4.4. Found: (*trans*)[K] C, 64.9; H, 4.6; (*cis*)[M] C, 65.0; H, 4.6.

When 2.3 g. of ethyl coumaronitrile-O-acetate at 90° was treated with 2 drops of concd. alcoholic sodium ethoxide, the temperature of the substance rose rapidly to 125°. There was obtained 1.8 g. of **2-carbethoxycoumaran-3-acetonitrile**, a colorless oil, b. p. 215–216° at 18 mm.

Anal. Calcd. for $C_{13}H_{13}NO_3$: C, 67.5; H, 5.6. Found[K]: C, 67.7; H, 5.6.

The *cis*-nitrile ester behaved in the same way when it was treated with sodium ethoxide, and the same product was obtained. In both cases the resulting cyclized nitrile ester was saponified, giving 2-carboxycoumaran-3-acetic acid, m. p. 170–172° alone or mixed with the acid obtained from ethyl 2-carbethoxycoumaran-3-acetate. In a larger scale experiment, 28 g. of a distilled mixture of ethyl coumaro- and ethyl coumarinonitrile-O-acetates gave 22.5 g. (80%) of pure 2-carbethoxycoumaran-3-acetonitrile.

Ethyl 2-Carbethoxy-7-methoxycoumaran-3-acetate (VIII, $R_1 = R_2 = R_3 = H$; $R_4 = OCH_3$)

8-Methoxycoumarin[17] (6.5 g.) was heated on a water-bath for five minutes with a solution of 0.85 g. of sodium

(15) Prepared according to the method of v. Auwers [*Ann.*, **393**, 352 (1912)] but from chloro- instead of bromoacetic ester.

(16) Lapworth and Baker, "Org. Syn.," Coll. Vol. I, p. 175, John Wiley and Sons, Inc., New York, N. Y., 1932.

(17) Dey and Kutti, *Proc. Natl. Inst. Sci. India*, **6**, 641 (1941); *Chem. Abs.*, **36**, 83 (1942).

in 12 ml. of dry ethanol. The resulting deep yellow gelatinous mass was then treated with 4.7 g. of ethyl chloroacetate, and boiling was continued for seventy-five minutes. The product was taken up in ether, washed with alkali, and distilled, giving 7.1 g. of **ethyl 3-methoxy-coumarate-O-acetate**, a colorless viscous oil, b. p. 220–224° at 10 mm.

Anal. Calcd. for $C_{16}H_{20}O_6$: C, 62.3; H, 6.5. Found[K]: C, 61.8; H, 6.6.

Saponification of the ester by boiling it for forty-five minutes with 10% potassium hydroxide gave **3-methoxy-coumaric acid-O-acetic acid**, colorless needles from 10% acetic acid, m. p. 227–228°. A cold solution of the acid in sodium carbonate decolorized permanganate immediately.

Anal. Calcd. for $C_{12}H_{12}O_6$: C, 57.1; H, 4.8. Found[K]: C, 57.1; H, 4.8.

When 2 g. of the ester was treated with a few drops of concd., alcoholic sodium ethoxide, it became brown, and the temperature of the mixture rose to 85°. There was obtained 1.3 g. of **ethyl 2-carbethoxy-7-methoxycoumaran-3-acetate**, a colorless oil, b. p. 208–211° at 10 mm.

Anal. Calcd. for $C_{16}H_{20}O_6$: C, 62.3; H, 6.5. Found[K]: C, 62.0; H, 6.5.

Saponification gave an oily acid (not analyzed) whose solution in sodium carbonate was stable to cold permanganate.

Ethyl 2-Carbethoxy-3,6-dimethylcoumaran-3-acetate
(VIII, $R_1 = R_4 = H$; $R_2 = R_3 = CH_3$)

4,7-Dimethylcoumarin[18] (52 g.) was boiled for fifteen minutes with 30 g. of sodium hydroxide in 150 ml. of water and 50 ml. of alcohol. To the resulting solution there was then added a solution of 36 g. of chloroacetic acid in 100 ml. of water containing 20 g. of sodium carbonate. Boiling was continued for one hour, 100 ml. of alcohol and water was removed by distillation, and the residue was acidified with hydrochloric acid. The mixture was then made slightly basic with sodium carbonate, and the unchanged dimethylcoumarin (19.2 g.) was removed. The acidic product (42.8 g.) was dried, boiled with 100 ml. of benzene, and crystallized from about one liter of water, giving 36 g. of **4,β-dimethylcoumarinic acid-O-acetic acid**, coarse needles that fell to a white powder on drying at 100°, then sintered at 180° and melted at 188–189° with gas evolution.

Anal. Calcd. for $C_{13}H_{14}O_5$: C, 62.4; H, 5.6; N. E., 125. Found[R]: C, 62.5; H, 5.6; N. E., 119.

A cold solution of 1.25 g. of the acid in dilute sodium carbonate decolorized 2.1 g. (calcd. 2.1 g.) of permanganate, The resulting **2-acetyl-5-methylphenoxyacetic acid** formed colorless prisms (0.95 g.) from water, m. p. 142–144°.

Anal. Calcd. for $C_{11}H_{12}O_4$: C, 63.4; H, 5.8. Found[R]: C, 63.1; H, 5.8.

A solution of 2.5 g. of **4,β-dimethylcoumarinic acid-O-acetic acid** in 25 ml. of water containing 1 g. of sodium hydroxide and 0.5 g. of Raney nickel was shaken with hydrogen at 25° and 740 mm. The product,[14] **4,β-dimethylmelilotic acid-O-acetic acid**, separated from water containing a little acetic acid in the form of white crystals (2 g.) that sintered at 155° and melted at 157–158°.

Anal. Calcd. for $C_{13}H_{16}O_5$: C, 61.9; H, 6.3. Found[K]: C, 62.0; H, 6.4.

A solution of 10 g. of 4,β-dimethylcoumarinic acid-O-acetic acid in 30 ml. of dry ethanol containing 2 ml. of concd. sulfuric acid was boiled for three hours and then poured into water and ether. Extraction with dilute sodium carbonate gave 1 g. of **ethyl 4,β-dimethylcoumarinic acid-O-acetate**,[19] colorless plates from benzene–ligroin, or flat needles from dilute alcohol, m. p. 96–97°.

Anal. Calcd. for $C_{15}H_{18}O_5$: C, 64.8; H, 6.5. Found[K]: C, 65.0; H, 6.5.

The neutral esterification product was **ethyl 4,β-dimethylcoumarinate-O-acetate**,[20] prisms (10.5 g., 86%) from ligroin, or flat needles from dilute alcohol, m. p. 48–51°, b. p. 196–197° at 9 mm.

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.7; H, 7.2. Found[R]: C, 67.0; H, 7.3.

Saponification of the ester with 10% aqueous potassium hydroxide yielded the parent acid.

When 2 g. of the ester at 53° was treated with a few drops of alcoholic sodium ethoxide, the mixture became red, and its temperature rose to 100°. Distillation gave 1.8 g. of **ethyl 2-carbethoxy-3,6-dimethylcoumaran-3-acetate**, b. p. 195–196° at 9 mm.

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.7; H, 7.2. Found[K]: C, 66.9; H, 7.4.

Saponification of the cyclized ester (1.2 g.) with 6% aqueous potassium hydroxide gave a sirupy acid which crystallized when it was rubbed with ether. This **2-carboxy-3,6-dimethylcoumaran-3-acetic acid** separated from water in the form of colorless prisms (0.9 g.) that sintered at 174° and melted at 177–178°; a mixture with the isomeric unsaturated acid melted at 155–163° with gas evolution. A solution in sodium carbonate was stable to warm permanganate.

Anal. Calcd. for $C_{13}H_{14}O_5$: C, 62.4; H, 5.6. Found[K]: C, 62.5; H, 5.9.

Ethyl 2-Carbethoxy-3,6-dimethylcoumaran-3,α-butyrate
(VIII, $R_1 = C_2H_5$, $R_2 = R_3 = CH_3$, $R_4 = H$)

Ten grams of 3-ethyl-4,7-dimethylcoumarin[21] was boiled for fifteen minutes with a solution of 6 g. of sodium hydroxide in 10 ml. of alcohol and 20 ml. of water; then a solution of 7.5 g. (1.6 eq.) of chloroacetic acid and 4 g. of sodium carbonate in 20 ml. of water was added, and the mixture was boiled for one hour. When it was worked up in the way described for 4,β-dimethylcoumarinic acid-O-acetic acid, the mixture furnished 7.7 g. of ethyldimethylcoumarin and 3.2 g. (23%) of **α-ethyl-4,β-dimethylcoumarinic acid-O-acetic acid**,[22] colorless prisms from 27% acetic acid, m. p. 161–162°.

Anal. Calcd. for $C_{15}H_{18}O_5$: C, 64.8; H, 6.5; N. E., 139. Found[K]: C, 64.9; H, 6.6; N. E., 139.

The acid was not reduced[14] when an aqueous solution of its sodium salt was shaken with hydrogen in the presence of Raney nickel. A solution of 1.4 g. of the acid in 10 ml. of 2.5% sodium carbonate decolorized 20 ml. of 5% permanganate (corresponding to about 2 eq. of oxygen); the crystalline product (0.6 g.) was 2-acetyl-5-methylphenoxyacetic acid, identical with the acid obtained from 4,β-dimethylcoumarinic acid-O-acetic acid; the mother liquors had a strong odor resembling that of pyruvic acid.

When 10 g. of α-ethyl-4,β-dimethylcoumarinic acid was boiled for three hours with 40 ml. of ethanol containing 2 ml. of sulfuric acid, it gave 7.7 g. of **ethyl α-ethyl-4,β-**

(18) Fries and Klostermann, *Ber.*, **39**, 874 (1906).

(19) In analogy with the known relative ease of esterification of saturated and α,β-unsaturated acids, this partial esterification product is formulated as having a free carboxyl attached to the unsaturated C atom.

(20) This ester could not be prepared in the very convenient way used for ethyl coumarate-O-acetate, directly from the coumarin, sodium ethoxide, and ethyl chloroacetate. The β-substituted coumarin is changed by sodium ethoxide into a complex product, and this change takes place much more readily than is indicated by the descriptions of Fries and co-workers [*Ann.*, **362**, 15 (1908); **379**, 95 (1911)], who have elucidated it.

(21) Fries and Klostermann, *Ann.*, **362**, 26 (1911).

(22) The proportion of unchanged coumarin to aryloxyacetic acid remained almost constant (between 72:28 and 77:23) in many other preparations in which as much as 4 equivalents of sodium chloroacetate was added all at once to the alkaline ethyldimethylcoumarin solution. However, the proportion was raised to 55:45 when 3 equivalents of sodium chloroacetate was added in five portions alternately with 4 equivalents of sodium hydroxide; the amounts used in each portion were calculated on the basis that the desired alkylation took place to the extent of 25% while 75% of the chloroacetate was converted into glycolate and 75% of the coumarin was regenerated from the disodium coumarinate.

dimethylcoumarinic acid-O-acetate,[19] colorless prisms from ether–ligroin, m. p. 73–74°.

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.7; H, 7.2. Found[K]: C, 66.3; H, 7.2.

The neutral esterification product was **ethyl α-ethyl-4,β-dimethylcoumarinate-O-acetate,**[23] a yellow oil (2.6 g., 22%), b. p. 203–205° at 13 mm.

Anal. Calcd. for $C_{19}H_{26}O_5$: C, 68.3; H, 7.8. Found[K]: C, 67.9; H, 7.9.

When 2 g. of this ester was warmed to 45° and treated with a few drops of alcoholic sodium ethoxide, the mixture became dark and its temperature rose to 95°. Distillation gave 1.75 g. of **ethyl 2-carbethoxy-3,6-dimethylcoumaran-3,α-butyrate**, a pale yellow oil, b. p. 207–210° at 13 mm.

Anal. Calcd. for $C_{19}H_{26}O_5$: C, 68.3; H, 7.8. Found[K]: C, 68.1; H, 7.9.

The acid obtained by saponifying the coumaran ester could not be obtained crystalline and was not analyzed. It was found to be stable to permanganate.

Ethyl 6-Methylspiro-[coumaran-3,1′-cyclopentane]-2,2′-dicarboxylate (VIII, $R_1 + R_2 = CH_2CH_2CH_2$, $R_3 = CH_3$, $R_4 = H$)

A mixture of 50 g. of *m*-cresol and 50 g. of ethyl cyclopentanone-2-carboxylate was treated with 200 g. of 90% sulfuric acid; during thirty minutes the temperature of the mixture rose from 20 to 40°. After it had stood overnight, the mixture was poured into water; the solid product was removed, washed with dilute sodium carbonate, and crystallized from alcohol. There was obtained 40 g. of **3,4-cyclopenteno-7-methylcoumarin**, colorless plates, m. p. 112–113° (reported[24] 105°); b. p. 215–216° at 13 mm.

Anal. Calcd. for $C_{13}H_{12}O_2$: C, 78.0; H, 6.0. Found[K]: C, 77.7; H, 6.2.

The coumarin (20 g.) was boiled for fifteen minutes with a solution of 12 g. of sodium hydroxide in 40 ml. of water and 20 ml. of alcohol. The resulting solution was treated with 10 g. of chloroacetic acid in 20 ml. of water containing 5.6 g. of sodium carbonate, and boiling was continued for twenty-five minutes. Worked up in the usual way, the mixture furnished 13 g. of unchanged 3,4-cyclopenteno-7-methylcoumarin and 9.0 g. of **4-methyl-α,β-trimethylenecoumarinic acid-O-acetic acid**, fine white crystals from dilute acetic acid, that sintered at 200° and melted at 216–217° with darkening and gas evolution.

Anal. Calcd. for $C_{15}H_{16}O_5$: C, 65.2; H, 5.8. Found[K]: C, 65.3; H, 6.0.

The acid was not reduced[14] when its solution in aqueous sodium hydroxide was shaken with hydrogen in the presence of Raney nickel. However, the alkaline solution rapidly decolorized permanganate.

A solution of 10 g. of the acid in 40 ml. of ethanol containing 2 ml. of sulfuric acid was boiled for three hours, giving 1.3 g. of oily acid-ester, and 10.2 g. (83%) of **ethyl 4-methyl-α,β-trimethylenecoumarinate-O-acetate**, colorless prisms from ligroin, m. p. 65–67°, b. p. 220–222° at 11 mm.

Anal. Calcd. for $C_{19}H_{24}O_5$: C, 68.7; H, 7.2. Found[K]: C, 68.3; H, 7.4.

Two grams of the diester was melted, supercooled to 50°, and treated with two drops of alcoholic sodium ethoxide. The mixture became dark and its temperature rose to 80°. Distillation gave 1.7 g. of **ethyl 6-methylspiro-[coumaran-3,1′-cyclopentane]-2,2′-dicarboxylate**, a colorless oil, b. p. 219–223° at 12 mm.

Anal. Calcd. for $C_{19}H_{24}O_5$: C, 68.7; H, 7.2. Found[K]: C, 68.9; H, 7.4.

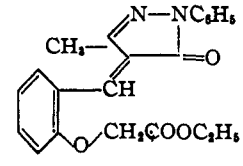
Saponification of the cyclized ester with 10% aqueous potassium hydroxide gave **6-methylspiro-[coumaran-3,1′-cyclopentane]-2,2′-dicarboxylic acid**, colorless hydrated prisms from water or dilute acetic acid, that melted indefinitely at 125–200°; the crystals effloresced when they were dried under reduced pressure at 115° for several hours and then melted at 208–210°.

Anal. Calcd. for $C_{15}H_{16}O_5 + H_2O$: H_2O, 6.1. Found[K]: H_2O, 6.25. Calcd. for $C_{15}H_{16}O_5$: C, 65.3; H, 5.8. Found[K]: C, 65.4; H, 6.1.

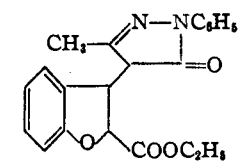
A solution of the acid in aqueous sodium carbonate was stable to permanganate.

Addendum

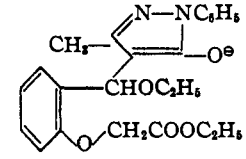
The following experiments indicate that the cyclization of X to XI does not take place. The polarization of the semicyclic double bond in X by two negative groups is much greater than is that of the α,β-double bond in a cinnamic ester derivative which does cyclize. The failure of X to cyclize is a result of the formation of a relatively stable anion (XII) in which the methylene adjacent to the carbethoxyl is protected from attack by the basic catalyst.



X



XI



XII

A mixture of 1.8 g. of 3-methyl-1-phenylpyrazolone-5 and 2.1 g. of ethyl *o*-formylphenoxyacetate was heated at 120–125° for one hour. Fractional crystallization from alcohol gave 0.8 g. of **o-carbethoxymethoxybenzalbis-4,4′-(3-methyl-1-phenylpyrazolone-5)**, colorless plates which were easily soluble in aqueous sodium hydroxide and which melted to an orange-red liquid at 204°.

Anal. Calcd. for $C_{31}H_{30}N_4O_6$: C, 69.1; H, 5.6. Found[K]: C, 69.1; H, 5.9.

There was also obtained 1.2 g. (33%) of **4-(o-carbethoxymethoxybenzal)-3-methyl-1-phenylpyrazolone-5 (X)**, orange-red needles, m. p. 122–123°, which were insoluble in aqueous-alcoholic sodium hydroxide. This compound was obtained in better yield (19 g.) by boiling a solution of 18 g. of methylphenylpyrazolone and 21 g. of ethyl formylphenoxyacetate in 50 ml. of acetic acid for one hour.

Anal. Calcd. for $C_{21}H_{20}N_2O_4$: C, 69.2; H, 5.5. Found[K]: C, 69.4; H, 5.7.

A suspension of 14.6 g. of X in 50 ml. of dry ethanol was treated with a solution of 1 g. of sodium in 15 ml. of ethanol. The solid dissolved immediately, giving a pale orange solution much lighter in color than a saturated neutral alcoholic solution of X. The addition of 2.5 ml. of acetic acid caused a rapid precipitation of 11.3 g. of X.

A similar mixture of X and alcoholic sodium ethoxide was boiled for three hours and allowed to stand for twenty-four hours at room temperature. Acidification then gave a resinous mixture from which there was isolated about 5 g. of X and 2 g. of the related benzalbispyrazolone.

Summary

It has been found that *o*-carbethoxymethoxy derivatives of ethyl cinnamate undergo cycliza-

(23) This ester could not be prepared directly from the coumarin (*cf.* footnote 20); 98% of the coumarin was recovered unchanged after it had been treated with alcoholic sodium ethoxide and ethyl chloroacetate.

(24) Ahmad and Desai, *Proc. Indian Acad. Sci.*, **5A**, 277 (1937); *Chem. Zentr.*, **108**, 229 (1937).

tion through an intramolecular reaction analogous to the well-known intermolecular Michael reaction. Unlike the latter, however, the intramolecular reaction is not inhibited by alkyl substituents on the α- and β-carbon atoms of the acceptor group, and it appears to go substantially to completion. The products are derivatives of 2-carboxycoumaran-3-acetic acid.

The reaction was devised and is being further studied as a possible route to synthetic compounds related to morphine.

MINNEAPOLIS, MINNESOTA RECEIVED DECEMBER 29, 1944

[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND MEDICINE, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, AND THE PRESBYTERIAN HOSPITAL, NEW YORK CITY]

Denatured Egg Albumin.[1] I. The Preparation and Purification of Crystalline Egg Albumin Denatured in Various Ways[2]

BY CATHERINE F. C. MACPHERSON AND MICHAEL HEIDELBERGER

Nowhere is the need for more precise information on denatured proteins more urgently implied than in the all-inclusive definition of denaturation given in the most recent review of the subject.[3] The prevailing utter lack of agreement as to the criteria of denaturation would seem to render a descriptive approach to the problem permissible, the more so as the original object of the present studies, the use of acid-denatured egg albumin (DnEa) as an intermediate, was thwarted by the extreme difficulty of obtaining successive preparations with reproducible properties. These difficulties, in part induced by the desire to avoid, as far as possible, degradation[4] of the egg albumin (Ea) used, necessitated a critical study of the preparation of acid-DnEa, and to this was added a similar investigation of other methods for the denaturation of Ea and the comparison and attempted correlation of the chemical, physical, and immunological properties of the products.

The criterion of denaturation employed was the insolubility, at the isoelectric point, of the product, DnEa, derived from the isoelectrically soluble native protein, hen egg albumin.

Qualitative immunological studies by Wu, Ten Broeck and Li[5] on egg albumin (Ea) denatured by acid, alkali, heat, and alcohol showed the denaturation products to be closely related to each other except that only alkali-DnEa did not react with antiserum to Ea and Ea did not react with antiserum to alkali-DnEa. Flosdorf and Chambers[6] found that denaturation by intense sound vibrations produced the same qualitative immunological changes as heating with acid or alkali. Mirsky[7] demonstrated that the same number of sulfhydryl groups was liberated in Ea denatured by urea, guanidine, Duponol P. C., heat, or shaking.

Each of these comparative studies dealt with only one property of DnEa and little attention was paid to the state of aggregation[8] or degradation[4] of the products, factors which not only would be expected to influence the chemical, physical, and immunological properties of DnEa but which also, if neglected, would cause confusion as to the changes attributable to denaturation alone. While it might be inferred from the few ultracentrifugal studies[9] on the particle weights of various kinds of DnEa that the molecules of these substances are associated or aggregated in water containing only just enough alkali to dissolve them, little, if anything, is known of the degree of association brought about by a particular denaturant or how this might be affected by variations in either the concentration of denaturant or the reaction time or temperature.

Experimental

Ea was prepared by the method of Kekwick and Cannan.[10] Four-times recrystallized Ea was air-dried and dialyzed against distilled water in the presence of toluene at 0–5° until no more sulfate was removed. Toward the end of these studies it was found that by allowing the salt-free isoelectric Ea solutions of varying opalescence to stand at 37° until all precipitate settled, any DnEa contained in them was removed, leaving a water-clear supernatant.

1. **Acid Denaturation of Ea.**—The denaturation of Ea by acid at a concentration of about 2.4 mg. Ea N per ml. was carried out by addition of 0.1 *M* HCl to bring the *p*H to 1.5 or 2.0. The final normality of the acid was usually about 0.05 or 0.025, respectively. The *p*H, determined with the glass electrode, remained constant during the reaction. In most instances the course of the de-

(1) A summary of this series of papers was read before a joint meeting of the New York and New Jersey Sections of the Society of American Bacteriologists on May 16, 1942, at Princeton, New Jersey.

(2) The work reported in this communication was carried out in part under the Harkness Research Fund of the Presbyterian Hospital and has been submitted by Catherine F. C. MacPherson in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University. Original manuscript received July 6, 1944.

(3) H. Neurath, J. P. Greenstein, F. W. Putnam and J. O. Erickson, *Chem. Rev.*, **34**, 157 (1944).

(4) Degradation is here defined as the splitting off of nitrogenous or sulfur-containing portions of the labile protein molecule.

(5) H. Wu, C. Ten Broeck, and E. P. Li, *Chinese J. Physiol.*, **1**, 277 (1927).

(6) E. W. Flosdorf and L. A. Chambers, *J. Immunol.*, **28**, 297 (1935).

(7) A. E. Mirsky, *J. Gen. Physiol.*, **24**, 725 (1941).

(8) Aggregation is defined as the linking of DnEa molecules, assuming M. W. DnEa = M. W. Ea = ca. 40,000, into aggregates or polymers. Opalescence and gel-formation were considered visual evidences of the process, useful for guidance during the preparative work. More substantial evidence is given in the text.

(9) (a) A. Rothen, *Annals N. Y. Acad. Sci.*, **43**, 229 (1942); (b) J. B. Nichols, THIS JOURNAL, **52**, 5176 (1930).

(10) R. A. Kekwick and R. K. Cannan, *Biochem. J.*, **30**, 232 (1926).