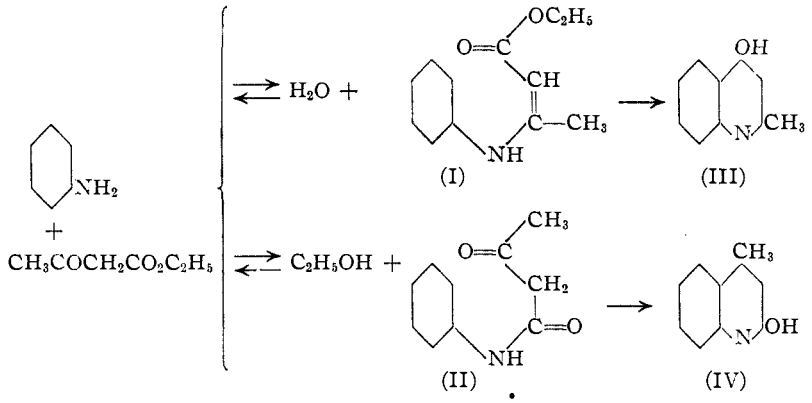
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF DUKE UNIVERSITY]

Reactions of β-Keto Esters with Aromatic Amines. Syntheses of 2- and 4-Hydroxyquinoline Derivatives[1]

BY CHARLES R. HAUSER AND GEORGE A. REYNOLDS

Earlier workers have shown that ethyl acetoacetate and aniline react at room temperatures to form ethyl β-anilinocrotonate (I)[2] or the anil, whereas, at 130–140°, acetoacetanilide (II) is produced.[3] On cyclization these products form 2-methyl-4-hydroxyquinoline (III) and 4-methyl-2-hydroxyquinoline (IV), respectively.



We have found that both crotonate and anilide formations are reversible; the crotonate is converted to the anilide by heating with an equivalent of water and a trace of acid[4] at 130–140°, whereas the reverse transformation takes place upon boiling the anilide with ethanol and Drierite. Furthermore, in contrast to ethyl acetoacetate, 2-ethylbutyl and *n*-amyl acetoacetates were found to react with aniline to form mainly the corresponding crotonates even at 130–140°. The crotonates and the anilide were identified by cyclizations to (III) and (IV), respectively.[5] Conceivably differences in the volatility of the by-products govern the course the reaction takes. If at all temperatures considered the equilibrium favors the crotonate, anilide formation in the case of the ethyl ester[6] could be accounted for, because ethanol, the by-product of the anilide formation, is more volatile than water, which would result from crotonate production.[7] On the other hand, one might assume that at 130–140° the equilibrium favors the anilide, and then the results could be understood on the basis of the lower volatility of 2-ethylbutyl and *n*-amyl alcohols as compared to water. The facts at hand do not permit stating which, if any, of these alternatives applies. However, since the products are readily interconvertible, it seems likely that the temperature dependence of the course of reaction is due to displacement of equilibrium rather than to the existence of two competing reaction paths with sufficiently differing temperature coefficients[8] of rate.

In Table I are given the over-all yields of various 2- and 4-hydroxyquinolines obtained from β-keto esters and aromatic amines employing various methods of preparation of the intermediate crotonates and anilides. The crotonates were generally prepared more conveniently by refluxing the reactants with Drierite in ethanol (Method B)[9,10] than by the older procedure (Method A) which requires a much longer time. However, Method B has not been satisfactory with ethyl benzoylacetate with which a modification of Method A was finally adopted (see note *e*, Table

(1) This work was supported by a grant from the Duke University Research Council.

(2) (a) Knorr, *Ber.*, **16**, 2593 (1883); (b) Conrad and Limpach, *Ber.*, **20**, 944 (1887); (c) Cavallito and Haskell, THIS JOURNAL, **66**, 1166 (1944); (d) Coffey, Thomson and Wilson, *J. Chem. Soc.*, 856 (1936).

(3) (a) Knorr, *Ann.*, **236**, 69 (1886); (b) Roos, *Ber.*, **21**, 624 (1888); (c) Knorr and Reuter, *Ber.*, **27**, 1169 (1894); (d) Knorr, *Ann.*, **236**, 74 (1894).

(4) The reaction failed in the absence of a trace of acid. This is not surprising since Coffey, Thomson and Wilson (ref. 2d) have shown that the formation of the crotonate requires a trace of acid, which ordinarily is present in commercial ethyl acetoacetate.

(5) The anilide was also identified (after recrystallization) by its melting point. The crotonates have been distilled at reduced pressures but, since the distillates deposited small amounts of diphenylurea, they were not analyzed. The diphenylurea was formed apparently from anilide, small amounts of which were evidently produced during the heating at 130–140° or during the distillation. Oppenheim and Precht (*Ber.*, **9**, 1098 (1876)) reported that heating the anilide with aniline produced diphenylurea and acetone.

(6) Also isopropyl acetoacetate appears to form the anilide at 130–140°, since a low yield of (IV) has been obtained on cyclizing the crude product. However, in attempts to reproduce this result, the only product isolated was diphenylurea which was formed presumably from the anilide (see note 5).

(7) Knorr (3d) reported that the anilide was obtained from aniline and ethyl acetoacetate when they were heated in a sealed tube at 150° for a long time. An attempt to reproduce this result was unsuccessful.

(8) This is evidently the case, for example, in the reaction of alkali with anti-benzaldoxime acetate which yields mainly the anti-benzaldoxime at 0° but mainly the corresponding nitrile at 30° or above; Hauser and Jordan, THIS JOURNAL, **57**, 2450 (1935).

(9) A little more than the calculated amount of Drierite required to remove the by-product, water, has generally been employed, but one-third of this amount has given, after cyclization of the crotonate, only a slightly lower yield of (III). It was found advisable to add a trace of acetic acid as catalyst.

(10) This procedure has also been satisfactory for the reaction of ethyl ethoxalylpropionate and aniline; cyclization of the resulting intermediate gave 3-methyl-4-hydroxy-2-carbethoxyquinoline, m. p. 177–179°, in 50% yield. However, the procedure using Drierite, as well as the original one, failed with ethyl ethoxalylpropionate and 2-nitro-4-methoxyaniline.

TABLE I

QUINOLINE DERIVATIVES FROM $RCOCH_2CO_2R'$ AND AROMATIC AMINES BY CYCLIZATION OF INTERMEDIATE CROTONATES AND ANILIDES OBTAINED BY VARIOUS METHODS

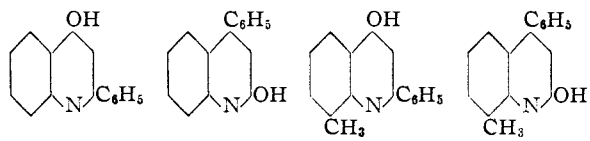
R	R'	Aromatic amine	Method	Substituted quinoline	M. p., °C.	Yield,[l] %
Methyl	Ethyl	Aniline	A	2-Methyl-4-hydroxy	229–230[a]	60
Methyl	Ethyl	Aniline	B	2-Methyl-4-hydroxy	229–230[a]	70
Methyl	*n*-Amyl[b]	Aniline	D	2-Methyl-4-hydroxy	229–230[a]	30
Methyl	2-Ethylbutyl[b]	Aniline	C, D	2-Methyl-4-hydroxy	229–230[a]	70
Methyl	Ethyl	*p*-Chloroaniline	B	2-Methyl-6-chloro-4-hydroxy	320–322[c]	68
Methyl	2-Ethylbutyl	*p*-Chloroaniline	C	2-Methyl-6-chloro-4-hydroxy	320–322[c]	70
Methyl	2-Ethylbutyl	*o*-Toluidine	C	2,8-Dimethyl-4-hydroxy	260–261[d]	68
Phenyl	Ethyl	Aniline	A[e]	2-Phenyl-4-hydroxy	253–254[f]	50
Phenyl	Ethyl	*o*-Toluidine	A	2-Phenyl-8-methyl-4-hydroxy	245–246[g]	38
Methyl	Ethyl	Aniline	D, E	4-Methyl-2-hydroxy	222–223[h]	50
Methyl	Ethyl	*o*-Toluidine	D, E	4,8-Dimethyl-2-hydroxy	217–218[i]	50
Phenyl	Ethyl	Aniline	F, G	4-Phenyl-2-hydroxy	259–260[j]	50
Phenyl	Ethyl	*o*-Toluidine	F, G	8-Methyl-4-phenyl-2-hydroxy	216–217[k]	38

[a] Conrad and Limpach, *Ber.*, **20,** 949 (1887). [b] Prepared by the method of Shivers, Dillon and Hauser, THIS JOURNAL, **69,** 119 (1947). [c] Kermac and Weatherhead, *J. Chem. Soc.*, 563 (1939). [d] Conrad and Limpach, *Ber.*, **21,** 524 (1888). [e] The reactants were allowed to stand ten days. The crotonate, obtained in 55% yield, melts at 92–93° (ref. 11). [f] Ref. 11. [g] Dziewonski, Moszew and Dortheimerowna, *Roczniki Chem.*, **12,** 925 (1932). [h] Camps, *Ber.*, **32,** 3230 (1901). [i] Ewins and King, *J. Chem. Soc.*, **103,** 107 (1913). [j] Ref. 12. [k] *Anal.* Calcd. for $C_{16}H_{13}NO$: C, 81.67; H, 5.61; N, 5.99. Found: C, 81.22; H, 5.80; N, 6.25. [l] Over-all yield base on the β-keto ester or aromatic amine.

I). Both Method A and B have failed with ethyl acetoacetate and *o*-nitroaniline or 2-nitro-4-methoxyaniline. Method C, which was used only with the higher alkylacetoacetates, is not generally as convenient as Method B. Method D, involving heating the reactant at 130–140°, produces the crotonates only with β-keto esters of sufficiently high boiling alcohols. The anilides were prepared from the ethyl β-keto esters either by Method D or by refluxing the reactants (Methods E and G).

It should be pointed out that, although cyclization of the crude crotonates prepared on a 0.1-mole scale by Method B gave quinolines of satisfactory purity, crude ethyl β-anilinocrotonate, prepared on a 0.5-mole scale, as a rule, gave products which were difficult to purify. When, however, the crotonate was purified by distillation under reduced pressure, the pure quinoline was always obtained.

Knorr reported that, like the crotonate, the anilide from aniline and ethyl benzoylacetate produces 2-phenyl-4-hydroxyquinoline (V) on cyclization.[11] He prepared the anilide by heating the reactants at 150° followed by treatment with dilute acid to hydrolyze ethyl β-phenylamidophenylacrylate, which was formed along with the anilide. However, we have found that heating the reactants at 150°, followed by recrystallization, produces essentially pure anilide, which, on cyclization, forms the expected 4-phenyl-2-hydroxyquinoline (VI). The two isomers, (V) and (VI), melt only a few degrees apart but a mixture of the two melts much lower and over a range. Moreover, treatment of (VI) with phosphorus oxychloride forms a chloro derivative the melting point of which is fifteen degrees higher than that reported for the chloro derivative of (V). Compounds (VII) and (VIII), which have been prepared from *o*-toluidine and ethyl benzoylacetate, melt almost thirty degrees apart. The present method of preparing 4-phenyl-2-hydroxyquinoline appears much more convenient than that described previously employing acetyl-*o*-amidobenzophenone.[12]



Experimental[13]

General Procedures.—The crotonates were prepared by one or more of the following procedures using 0.1 mole each of β-keto ester and aromatic amine. Method A: the reactants were allowed to stand at room temperatures (20–30°) either alone for four to five days[2a,b,c] or in the presence of a trace of hydrochloric acid (or aniline hydrochloride) in a vacuum desiccator over concentrated sulfuric acid for one to three days.[2d] Method B: to the reactants was added 30–40 ml. of commercial absolute ethanol, about 35 g. of Drierite, and three or four drops of glacial acetic acid. The resulting mixture was refluxed on the steam-bath for three to four hours. The Drierite was filtered off and the ethanol was distilled at slightly above room temperatures by means of a water aspirator. Method C: the reactants were heated at 95–100° in an oil-bath for three to four hours in the presence of about 10 g. of Drierite and the Drierite then filtered off. Method D (applicable to β-keto esters of higher alcohols): the reactants were heated in an open Erlenmeyer flask or beaker for three or four hours in an oil-bath at 130–140° and then cooled to room temperatures.

The crude crotonate was added as rapidly as possible to 100 ml. of stirred refluxing (250–260°) Dowtherm[14] contained in a 200-ml. three-neck round-bottom flask equipped with a mercury-sealed stirrer and condenser. After fifteen

(11) Knorr, *Ann.*, **245,** 378 (1888).

(12) Camps, *Arch. Pharm.*, **237,** 683 (1899).

(13) Analyses by Oakwold Laboratory, Alexandria, Virginia.

(14) This cyclization has been effected previously under other conditions; Cavallito and Haskell (ref. 3c) effected the reaction in mineral oil.

to twenty minutes the stirring was stopped and the mixture allowed to cool, a light yellow solid usually separating. Approximately 200 ml. of Skellysolve "B" was then added. After shaking, the solid 4-hydroxyquinoline derivative was filtered off, washed several times with Skellysolve and recrystallized from water in the case of 2-methyl-4-hydroxyquinoline and from a mixture of water and ethanol in the other cases.

Acetoacetanilides were prepared from 0.1 mole each of ethyl acetoacetate and aromatic amine either by Method D described above or by refluxing the reactants three to four minutes[15] (Method E). The solid, obtained on cooling the mixture, was recrystallized from acetic acid and water and then from ethanol and water yielding acetoacetanilide (m. p. 82–83°)[3a] in 52% yield and acetoaceto-*o*-toluidide (m. p. 107–108°)[15] in 55% yield.

Benzoylacetanilides were prepared from 0.1 mole each of ethyl benzoylacetate and aromatic amine either by heating the reactants at 150° for five hours (Method F) or by refluxing the mixture for fifteen minutes (Method G). After recrystallization as described for acetoacetanilide, benzoylacetanilide (m. p. 107–108°)[11] was obtained in 50% yield, and benzoylaceto-*o*-toluidide (m. p. 130–131°) in a 65% yield.

Anal. Calcd. for $C_{16}H_{15}NO_2$: C, 75.82; H, 5.96. Found: C, 75.51; H, 5.72.

The anilides were cyclized in concentrated sulfuric acid at 80–90° as described in "Organic Syntheses"[16] or by heating on the steam-bath for fifteen minutes. The 2-hydroxyquinoline derivatives were recrystallized from a mixture of water and ethanol. An attempt to cyclize acetoacetanilide (II) in Dowtherm at 250–260° as described above for the crotonate was unsuccessful.

The yields and the melting points of the quinoline derivatives are given in Table I. Admixture of the various samples of the same derivative showed no depression in melting point, but admixture of isomeric 2- and 4-hydroxyquinolines depressed the melting point.

2-Methyl-4-hydroxyquinoline (0.5 mole scale).—A mixture of 46.5 g. (0.5 mole) of aniline, 65 g. (0.5 mole) of ethyl acetoacetate, 100 ml. of commercial absolute ethanol, 135 g. of Drierite, and 1 ml. of glacial acetic acid was refluxed on the steam-bath for four hours. After removing the Drierite and the solvent, the residue was fractionated through a 30-cm. Vigreux column yielding 58 g. (57%) of ethyl β-anilinocrotonate, b. p. 155° at 10 mm.[17] The crotonate was cyclized in 200 ml. of Dowtherm yielding 38 g. (50%) of 2-methyl-4-hydroxyquinoline (m. p. 229–230°).

4-Phenyl-2-chloroquinoline.—A mixture of 10 g. (0.045 mole) of 4-phenyl-2-hydroxyquinoline (m. p. 259°) and 30 ml. of phosphorus oxychloride was heated in an oil-bath at 120° for two hours, the excess oxychloride distilled under reduced pressure and the light brown viscous oily residue poured onto ice. After standing in the refrigerator for one day the solidified oil was recrystallized from absolute ethanol yielding 10 g. (93%) of white crystals of 4-phenyl-2-chloroquinoline, m. p. 87–88°.

Anal. Calcd. for $C_{15}H_{10}NCl$: C, 75.15; H, 4.21; N, 5.86; Cl, 14.8. Found: C, 74.88; H, 4.70; N, 6.12; Cl, 14.68.

Conversion of Crotonate to Anilide.—To 0.1 mole of ethyl β-anilinocrotonate, b. p. 155° at 10 mm., was added 2 g. of water and five drops of concentrated hydrochloric acid and the mixture stirred and heated in an open flask in an oil-bath at 130–140° for three or four hours. The resulting crude anilide was cyclized[16] to form 4-methyl-2-hydroxyquinoline, m. p. 222–223°, in 35% over-all yield.

Conversion of Anilide to Crotonate.—A mixture of 0.1 mole of acetoacetanilide (m. p. 82–83°), 30 ml. of commercial absolute ethanol and 30 g. of Drierite was refluxed four hours and the Drierite then filtered off. After distilling the solvent, the residue was fractionated giving a 50% yield of ethyl β-anilinocrotonate (b. p. 139–143° at 6 mm.) which was cyclized in Dowtherm to 2-methyl-4-hydroxyquinoline, m. p. 229–230°, in 39% over-all yield from the anilide.

Summary

1. The factors governing the formation of crotonates and anilides from β-keto esters and aromatic amines have been considered.

2. Crotonates and anilides, prepared by various methods, have been cyclized to form 4- and 2-hydroxyquinolines, respectively.

3. In contrast to reports in the literature the anilide from ethyl benzoylacetate and aniline was found to form 4-phenyl-2-hydroxyquinoline on cyclization.

(15) Ewins and King, *J. Chem. Soc.*, **103**, 104 (1913), effected the reaction in one and one-half minutes.

(16) Lauer and Kaslow, "Organic Syntheses," Vol. 24, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 68.

(17) Distillation of the residue at a bath temperature of 120° until the forerun was removed and then at 140–160° gave ethyl β-anilinocrotonate, b. p. 128–130° at 2 mm., in 60–70% yield.

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The Bacterial Activity of "Racemized Casein," Caseose, and the Four Diastereoisomeric Leucylleucines[1,2]

BY SIDNEY W. FOX, YUTAKA KOBAYASHI,[3] SAMUEL MELVIN[3] AND FREDERICK N. MINARD[4]

Several of the antibiotics, particularly penicillin and gramicidin, are notable for their content of D-amino acid residues. In the case of penicillin, the D-amino acid residue is one of a number of critical structural features, since the L-analog is without activity.[5] D-Amino acids have been shown experimentally to inhibit bacterial growth[6,7] at relatively high concentrations. Recent reports, however, indicate medical utility of some of the simple amino acids, when used in relatively large amounts, in the control of infection.[8] In view of the above observations it is of interest to determine the antibacterial activity of structures re-

(1) Journal Paper No. J-1514 of the Iowa Agricultural Experiment Station, Project 897, in coöperation with the Veterinary Research Institute, and Project 980.

(2) The experiments with "racemized casein" were described before the American Society of Biological Chemists, May, 1947, at Chicago.

(3) This work was supported in part by the Industrial Science Research Institute of Iowa State College.

(4) Upjohn Company Fellow.

(5) du Vigneaud, Carpenter, Holley, Livermore and Rachele, *Science*, **104**, 431 (1946).

(6) Fox, Fling and Bollenback, *J. Biol. Chem.*, **155**, 465 (1944).

(7) Kobayashi, Fling and Fox, *ibid.*, **174**, 391 (1948).

(8) Mario, *Minerva med.*, **38**, I, 578 (1947).