[COMMUNICATION FROM THE INSTITUTE OF CHEMISTRY OF THE FACULTY OF SCIENCES, UNIVERSITY OF BELGRADE]

Reactions of Bisamides. VI. Synthesis of β-Aryl-α,β-Diaminopropionic Acids

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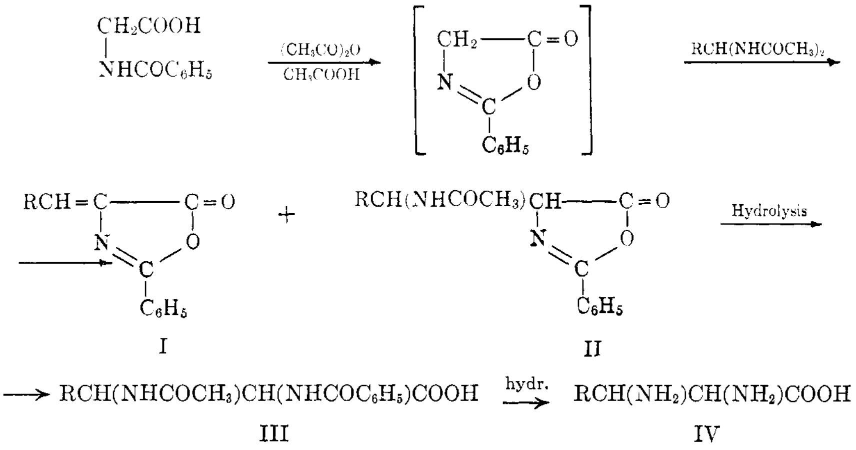
Received August 8, 1955

By condensing aromatic N,N-bisamides with hippuric acid in the presence of acetic anhydride and glacial acetic anhydride, α,β-diacylamino-β-arylpropionic acids are obtained as main products; in some cases the corresponding saturated azlactones are also formed. In these reactions two acids are always produced (*threo* and *erythro* racemic acids) as anticipated theoretically. These acids, by further acid hydrolysis, readily yield the corresponding free α,β-diamino acids. The configurations of α,β-diacyl-α,β-diaminopropionic acids and their azlactones are proposed and discussed.

Continuing our studies of the reactions of bisamides,[2-6] we have investigated the condensations of arylidene-N,N-bisamides with hippuric acid in the presence of glacial acetic acid and acetic anhydride. It has been found:

1. That these condensations proceed according to the following scheme, *i.e.*, that azlactones of β-arylacrylic acids I, azlactones of α, β-diacylaminopropionic acids II, and the corresponding acids III, are obtained, which can be hydrolyzed to the free α,β-diamino-β-arylpropionic acids IV;



I

II

$$\longrightarrow \mathrm{RCH(NHCOCH_3)CH(NHCOC_6H_5)COOH} \xrightarrow{\text{hydr.}} \mathrm{RCH(NH_2)CH(NH_2)COOH}$$

III IV

2. That these reactions are more complex than the previously described condensations,[2-6] namely that they proceed in both directions, I and II, *i.e.* III, and that they depend to a greater extent on experimental conditions.

First the condensations of benzylidenebisacetamide with hippuric acid will be discussed, as being characteristic for this type of condensation. Bisamides substituted in the benzene ring, however, react in a slightly different manner.

By crystallization from ethanol two substances are isolated from the reaction mixture: a yellow compound V, in 10% yield (see Table I), identified as 2-phenyl-4-benzylidene-5-oxazolone[8-11] and the colorless azlactone of α-benzoylamino-β-acetylamino-β-phenylpropionic acid VI (50-60%), whose structure was established on the basis of the following reactions:

a. By saponification with dilute solutions of sodium or potassium hydroxide, the azlactone VI furnishes the corresponding acid VII, which undergoes ring closure to the starting azlactone VI when heated with acetic anhydride;

b. The acid VII gives with diazomethane the methyl ester VIII;

c. The azlactone VI and aniline combine to yield the corresponding anilide IX;

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[1] Abstracted in the main from portions of the Ph.D. Dissertation of M.S., Institute of Chemistry of the Faculty of Sciences, University of Belgrade, Yugoslavia, 1954.

[2] Stefanović and Bojanović, *J. Org. Chem.*, **17**, 816 (1952).

[3] Stefanović, Bojanović, and Sirotanović, *J. Org. Chem.*, **17**, 1110 (1952).

[4] Stefanović and Stefanović, *J. Org. Chem.*, **17**, 1114 (1952).

[5] Stefanović and Nikić, *J. Org. Chem.*, **17**, 1305 (1952).

[6] Stefanović, Mihajlović, and Stefanović, *J. Org. Chem.*, **18**, 1467 (1953).

[7] Karrer and Bussmann, *Helv. Chim. Acta*, **24**, 645 (1941).

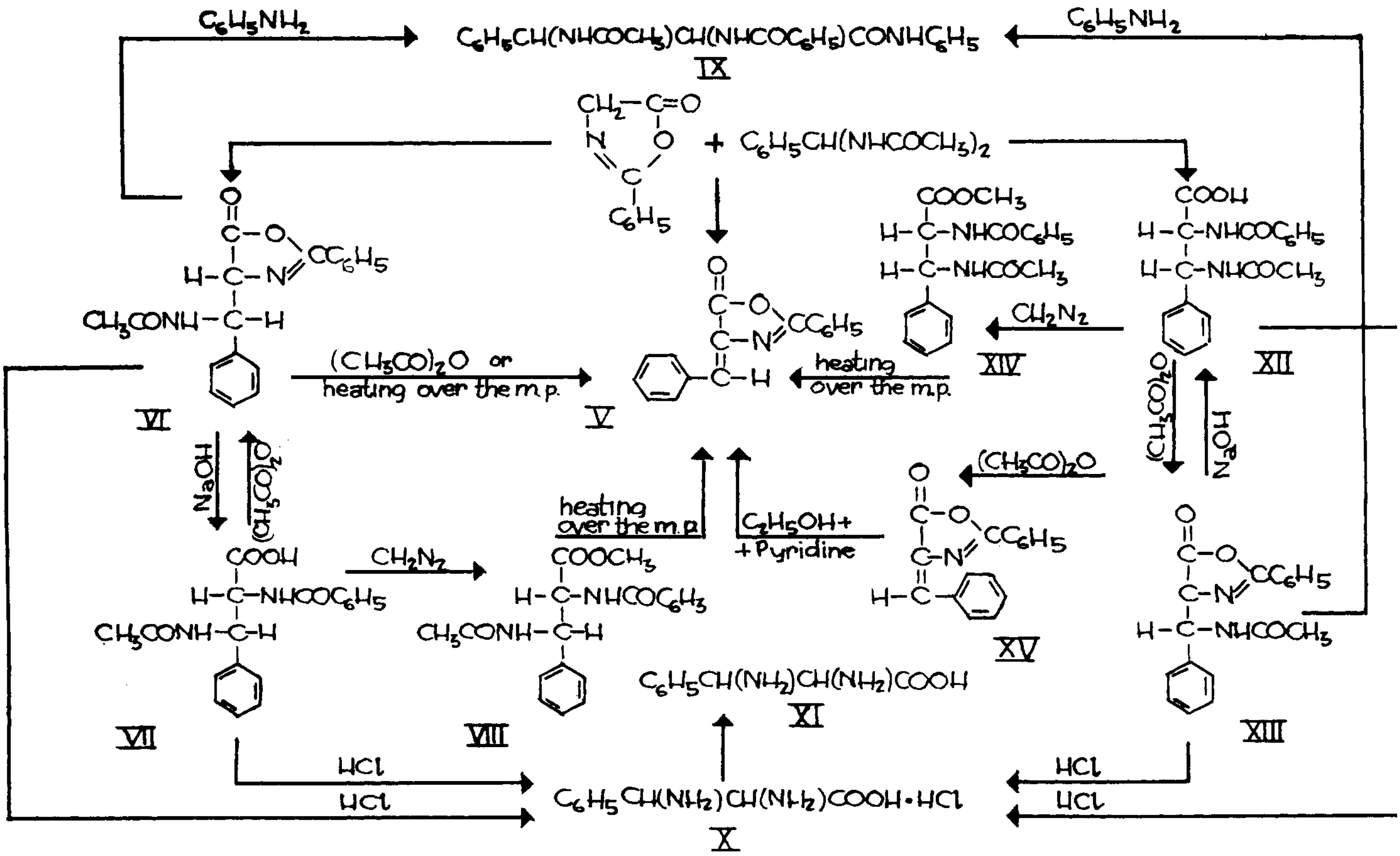
[8] Arylidene oxazolones (azlactones of β-arylacrylic acids) are readily obtained by the condensation of aryl aldehydes with hippuric acid in the presence of acetic anhydride and anhydrous sodium acetate. (Regarding Azlactones see: (a) Carter, *Org. Reactions*, **3**, 198-239 (1949). (b) *The Chemistry of Penicillin*, Princeton University Press, 1949, Princeton, N. J., pp. 830-848.

[9] Plöchl, *Ber.*, **16**, 2815 (1883).

[10] Erlenmeyer, *Ann.*, **275**, 1 (1893).

[11] Gillespie and Snyder, *Org. Syntheses*, **14**, 80 (1934).

TABLE I



d. On heating above their melting point or on prolonged heating with acetic anhydride all three compounds, the azlactone VI, the acid VII, and its methyl ester VIII, are converted to 2-phenyl-4-benzylidene-5-oxazolone V;

e. By acid hydrolysis of the azlactone VI, as well as of the acid VII, the monohydrochloride of α,β-diamino-β-phenylpropionic acid X is obtained which, when treated with silver oxide, gives the corresponding free acid XI.

After the separation of the unsaturated and saturated azlactones (V and VI), a third brown substance, which could not be purified by crystallization or chromatography, remains in the ethanol filtrate. On prolonged heating with water or by the action of dilute solutions of sodium or potassium hydroxide in the cold, it was converted to a new acid whose analysis corresponded to α-benzoylamino-β-acetylamino-β-phenylpropionic acid. From the reactions described below it appears that this acid is isomeric with the compound VII, and has therefore the structure XII:

a. When heated for a short time with acetic anhydride, the acid XII furnishes the corresponding azlactone XIII, which on saponification is again transformed to the acid XII;

b. The azlactone XIII gives with aniline an anilide which is identical with the anilide obtained from the azlactone VI;

c. Diazomethane reacts with the acid XII and yields a colorless substance XIV, which according to analysis is probably a mixture of the methyl ester and the azlactone XIII. (It appears that diazomethane in this case acts as a dehydrating agent producing, in part, ring closure of the acid XII);[7]

d. By heating the acid XII with acetic anhydride a bright yellow compound is obtained, which on heating in a mixture of alcohol and pyridine gives 2-phenyl-4-benzylidene-5-oxazolone V.[9–11] The yellow compound itself is believed to be a geometrical isomer of the unsaturated azlactone V, having the structure XV;

e. On being heated above their melting point, the acid XII, its methyl ester XIV, and the corresponding azlactone XIII are converted to 2-phenyl-4-benzylidene-5-oxazolone V;

f. By acid hydrolysis of the acid XII and azlactone XIII, the monohydrochloride of α,β-diamino-β-phenylpropionic acid is obtained. This compound is identical with the derivative prepared from the azlactone VI and acid VII.

THE CONFIGURATION OF THE AZLACTONES AND ACIDS

As already mentioned, the acid VII gives on prolonged heating with acetic anhydride 2-phenyl-4-benzylidene-5-oxazolone V, whereas the acid XII, under the same conditions, is converted to the unsaturated azlactone XV, which, in turn, on heating in ethanol-pyridine yields the stable isomer V.

These facts are in agreement with the *cis-trans* isomerism of unsaturated azlactones as suggested by Erlenmeyer and Bergman[12-14] and confirmed by Carter and Stevens,[15-16] who, in 1940, isolated for the first time the *cis* and *trans* isomers of α-benzoylaminocrotonic acid and also the isomers of 2-phenyl-4-benzylidene-5-oxazolone (the stable form melting at 164–166°, and the labile form at 146–148°), which were obtained by treating α-benzoylamino-β-methoxy-β-phenylpropionic acid with acetic anhydride.

The saturated acids (VII and XII) differ not only in their melting points, but also in other physical properties (solubility, crystalline form, etc.). They have two asymmetric carbon atoms and can exist in two racemic and four optically active forms. By resolution of the racemic acids into the optically active forms and by elimination of the acetyl and benzoyl groups followed by treatment of the free diamino acids with nitrous acid, the corresponding hydroxy acids of known configuration should be obtained.[17-18]

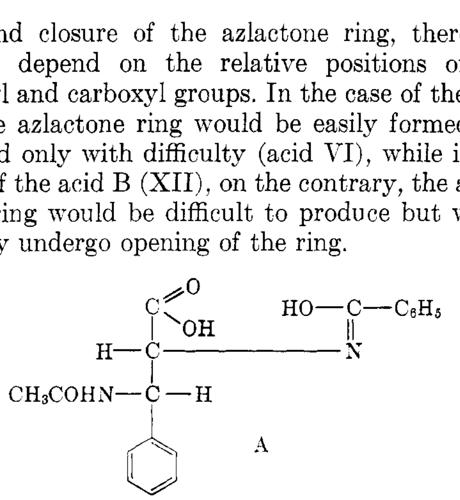
The possibility of the transformation of one isomer into the other in the course of these reactions cannot be excluded.

Our attempts to resolve these acids into optically active forms gave negative results. With strychnine, brucine, quinine, and quinidine we obtained in each case one crystalline substance in almost quantitative yield, wherefrom optical antipodes could not be isolated. By hydrolysis of various crops of these crystals the starting racemic acid was always recovered.

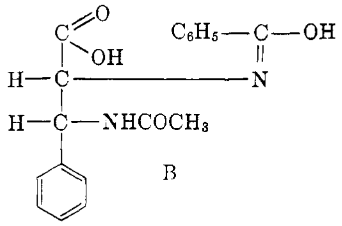
However, certain conclusions may be drawn about the configurations of the acids (VII and XII) from the following data:

a. The acids VII and XII, under the same experimental conditions, easily undergo ring closure giving the saturated azlactones VI and XIII, but the rate of hydrolysis of these azlactones differs greatly. Thus, the azlactone VI, which corresponds to the acid VII, after four hours of heating with water is only slightly hydrolyzed (about 0.40%), whereas the azlactone XIII, corresponding to the acid XII, hydrolyzes fifty times as fast (after 4 hours about 20%).

Assuming that closure of the azlactone ring occurs through the *enol* form of the benzoylamino group of these acids, *cis-trans* isomerism could be envisaged, *i.e.*, during the cyclization reaction the acids A and B could be formed. The ease of opening and closure of the azlactone ring, therefore, would depend on the relative positions of the phenyl and carboxyl groups. In the case of the acid A, the azlactone ring would be easily formed but opened only with difficulty (acid VI), while in the case of the acid B (XII), on the contrary, the azlactone ring would be difficult to produce but would readily undergo opening of the ring.



A



B

However, no difference could be observed in the rate of cyclization (azlactone formation), probably because these reactions are conducted under more drastic conditions than hydrolysis of the azlactone ring.

b. From the *cis-* and *trans*-arylideneoxazolones V and XV, which are obtained on longer heating of the acids VI and XII with acetic anhydride, the configurations of the acids can be deduced in the following way:

If one assumes that the stable unsaturated azlactone V is the *trans* isomer, the corresponding acid VII, from which V can be directly obtained, would have the stable *threo* configuration. On the other hand, the acid XII would have the *erythro* configuration corresponding to the unstable *cis*-azlactone XV. It follows, therefore, that the azlactone VI, as well as the acid VII, belongs to the *threo* series, whereas the acid XII and its saturated azlactone XIII have the *erythro* configuration. A direct proof for this assumption, however, must await further experimental evidence.

CONDENSATIONS OF ARYLIDENE-N,N-BISAMIDES SUBSTITUTED IN THE BENZENE RING

Beside benzylidene-N,N-bisamide other substituted bisamides, such as 4-methyl-, 3,4-methylenedioxy-, 2-methoxy-, and 4-methoxybenzylidene-N,N-bisacetamides have been condensed with hippuric acid. In each case two α-benzoylamino-β-acetylamino-β-arylpropionic acids were isolated, together with small amounts of the unsaturated azlactones. The saturated azlactones corresponding to these acids were not obtained, probably because they readily undergo hydrolysis with opening of the azlactone ring. Cyclization of the acids

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(12) Erlenmeyer, Matter, Stadlin, and Wittemberg, *Ann.*, **337**, 271 (1904).

(13) Erlenmeyer and Kunlin, *Ber.*, **35**, 384 (1902).

(14) Bergman and Stern, *Ann.*, **448**, 20 (1926).

(15) Carter and Stevens, *J. Biol. Chem.*, **133**, 117 (1940).

(16) Carter and Riser, *J. Biol. Chem.*, **139**, 255 (1941).

(17) Rieber, *Ber.*, **48**, 823 (1915).

(18) Rieber and Berner, *Ber.*, **50**, 893 (1917).

to the saturated azlactones was also attempted by treatment with acetic anhydride, but in each case only the unsaturated azlactones were obtained.

2-Methoxybenzylidenebisacetamide, however, behaves differently and furnishes a saturated azlactone, whose formation is due, probably, to the well known *ortho*-effect.

CONDENSATIONS OF *ortho-*, *meta-*, AND *para-*NITROBENZYLIDENEBISAMIDES

Ortho- and *para*-nitrobenzylidene-N,N-bisacetamides do not condense with hippuric acid (80% of the unreacted bisamide was recovered, beside resinous material which could not be purified), whereas the *meta* derivative gives at higher temperature the azlactone of α-benzoylamino-β-(3-nitrophenyl)acrylic acid, as well as the acid itself. The acid and the azlactone are known compounds.[19]

The α,β-diamino-β-arylpropionic acids which have been described in this work are new compounds, with the exception of α-benzylamino-β-amino-β-phenylpropionic acid which had been previously obtained in very poor yields by addition of hydroxylamine to the ethyl ester of α-benzoylaminocinnamic acid (Posner's method).[20] However, attempts have been made in the past to obtain aromatic α,β-diamino acids. Thus:

a. By the addition of ammonia (or methylamine) to the ethyl ester of α-bromocinnamic acid the corresponding α,β-diamino acids could not be isolated, whereas by treatment with dimethylaniline and piperidine the bisdimethylamino- and bispiperidino-β-phenylpropionic acids were obtained.[21–22]

b. By the action of liquid and gaseous nitrogen dioxide on the ethyl ester of cinnamic acid a mixture of nitronitrate- and dinitroderivatives was produced. On reduction α,β-diamino- and hydroxyaminohydrocinnamic acids were formed but could not be isolated from the reaction mixture.[23]

EXPERIMENTAL[24]

The melting points are uncorrected.

1. *Azlactone* (VI) *of α-benzoylamino-β-acetylamino-β-phenylpropionic acid.* In a 250-cc. three-necked flask equipped with a mechanical stirrer and air condenser, 35.8 g. ($^1/_5$ mole) of hippuric acid was dissolved in 60 g. (1 mole) of glacial acetic acid and 20.4 g. ($^1/_5$ mole) of acetic anhydride, with vigorous stirring at 110° (oil-bath). Benzylidenebisacetamide (41.2 g., $^1/_5$ mole) then was introduced and after it had been dissolved, heating and stirring were continued for 75 minutes. The cooled solution was poured into 1 l. of ice-cold water and the resulting mixture was stirred with occasional replacement of water (by decanting the water from the mixture and adding ice-cold water), until the mass had solidified. After standing for 24 hours in the cold, filtering, and air-drying, 60 g. of a crude, yellowish-brown product was obtained. Dissolved in 500 cc. of 96% ethanol it afforded, on cooling, 5 g. (10%) of a yellow crystalline compound, m.p. 160–163°. This compound proved identical with *2-phenyl-4-benzylidene-5-oxazolone* (V), m.p. 166–167°, obtained by the condensation of benzaldehyde with hippuric acid in the presence of acetic anhydride and anhydrous sodium acetate.[9–11] The same oxazolone (V) was prepared by dissolving 7.2 g. of hippuric acid ($^1/_{25}$ mole) in 12.3 g. (3 × $^1/_{25}$ mole) of acetic anhydride at 120° with mechanical stirring, introducing 8.4 g. ($^1/_{25}$ mole) of benzylidenebisacetamide and 2.9 g. ($^1/_{25}$ mole) of anhydrous sodium acetate, and heating the solution for 3 hours. The resulting mixture was treated as above and it afforded 6.3 g. (62%) of the oxazolone (V), m.p. 165–166°.

Anal. Calc'd for $C_{16}H_{11}NO_2$: N, 5.62. Found: N, 5.86.

After the azlactone, m.p. 165–166°, had been removed, the ethanol filtrate was concentrated *in vacuo* to about 250 cc. and allowed to stand at 0° for 48 hours. The colorless crystalline substance which separated was filtered off and washed with a little cold ethanol. The yield of the crude compound, m.p. 204–205°, was 37.5 g. or 61%. After several crystallizations from ethanol the m.p. was raised to 207°. The product is sparingly soluble in ether and benzene, insoluble in water, and readily soluble in hot ethanol and acetone. It was identified as VI.

Anal. Calc'd for $C_{18}H_{16}N_2O_3$: C, 70.13; H, 5.19; N, 9.09. Found: C, 70.41; H, 5.40; N, 9.21.

2. *α-Benzoylamino-β-acetylamino-β-phenylpropionic acid* (XII). After the isolation of the unsaturated and saturated azlactone (V and VI), the remaining ethanol solution was concentrated *in vacuo.* On trituration with ice-cold water the sirupy residue solidified, giving 10 g. of brown-yellowish product melting at 70–160°. This was hydrolyzed by refluxing for 2 hours with 250 cc. of water, decanting the solution and repeating the procedure with the unhydrolyzed portion. The combined aqueous solutions were acidified with dilute hydrochloric acid to Congo Red and allowed to cool. The yellowish solid collected by filtration after air-drying and washing with ethyl acetate, was recrystallized from ethanol yielding 5 g. (8%) of pure XII, m.p. 238°. The acid is insoluble in ether, ethyl acetate, and water; soluble in hot ethanol and acetone. It is readily soluble in a 3% aqueous solution of sodium bicarbonate from which it is precipitated on acidification.

The same acid XII was also obtained by saponification of the crude starting product dissolved in ethanol, with 10% NaOH in the cold.

Anal. Calc'd for $C_{18}H_{18}N_2O_4$: C, 66.26; H, 5.52; N, 8.59. Found: C, 66.46; H, 5.72; N, 8.35.

The molecular weight was determined by titration in ethanol solution. Calc'd: 326. Found: 330.

3. *Methyl ester* (XIV) *of α-benzoylamino-β-acetylamino-β-phenylpropionic acid.* A solution of diazomethane in ether was added dropwise to the acid XII, contained in a flask equipped with a double-surface condenser and dropping-funnel, until the solution in the flask turned yellow. The solid (2.8, 90%) was filtered off, washed with a little ether, and recrystallized from 50% ethanol, yielding a product, m.p. 245–246°. Figures obtained by analysis of this product did not correspond exactly to the percentage composition of the methyl ester XIV, probably because some azlactone XIII was also formed by the action of diazomethane on the acid XII, which could not be removed by crystallization from ethanol.[7]

The ester was readily hydrolyzed with 30% NaOH at room temperature to the starting acid XII, whereas by heating above its melting point it was converted to V.

Anal. Calc'd for $C_{19}H_{20}N_2O_4$: C, 67.04; H, 5.88; N, 8.23. Found: C, 68.65, 68.50; H, 5.90, 5.95; N, 8.82.

4. *α-Benzoylamino-β-acetylamino-β-phenylpropionic acid* (VII). A solution of the azlactone VI in 50 cc. of 96% ethanol was mixed with 50 cc. of 10% aqueous sodium

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(19) Douglas and Gulland, *J. Chem. Soc.*, 2893 (1931).

(20) Posner, *Ann.*, **389**, 1 (1912).

(21) Roberts, *J. Chem. Soc.*, 1169 (1936).

(22) Roberts, *J. Chem. Soc.*, 963 (1938).

(23) Vasiljev and Vovtchenko, *Zhur. Obshchei Khim.* (*J. Gen. Chem.*), **20**, 1236 (1950); *Chem. Abstr.*, **45**, 1541 (1951).

(24) Prior to analysis all compounds were dried in a vacuum desiccator over KOH and P_2O_5.

hydroxide. After standing at room temperature for 3 days, the solution was neutralized with 10% hydrochloric acid to Congo Red, diluted with 50 cc. of water, and filtered off. Air-drying and crystallization from ethanol gave 4 g. (76%) of the acid VII, m.p. 201°. This colorless acid is readily soluble in hot ethanol, less soluble in ethyl acetate, insoluble in ether and water. From its solution in 3% aqueous sodium bicarbonate it can be recovered by acidification with dilute mineral acids, in the form of a voluminous precipitate which is difficult to filter.

Anal. Calc'd for $C_{18}H_{18}N_2O_4$: C, 66.26; H, 5.52; N, 8.69. Found: C, 66.16; H, 5.75; N, 8.53.

5. *Methyl ester* (VIII) *of α-benzoylamino-β-acetylamino-β-phenylpropionic acid.* The acid VII (3 g.) was treated with diazomethane as described under 3. The solid ester VIII (3 g., 95%) was filtered, washed with a little ether and recrystallized from 50% ethanol. It melts at 110–115°, solidifies and melts again with decomposition at 234–236°. It is soluble in ethanol, acetone, and benzene in the cold, less soluble in ether, insoluble in water. By heating above its melting point the ester VIII gave V, and by saponification with aqueous sodium hydroxide it afforded the starting acid VII.

Anal. Calc'd for $C_{19}H_{20}N_2O_4$: C, 67.06; H, 5.88; N, 8.23. Found: C, 67.24; H, 6.08; N, 8.69.

6. *Azlactones* VI, XIII *from α-benzoylamino-β-acetylamino-β-phenylpropionic acids* VI, XII. The acid VII (5 g.) was heated with 50 cc. of acetic anhydride on the water-bath, the anhydride was removed under reduced pressure, and the residue, after it had solidified by addition of cold water, was recrystallized from ethanol, yielding 4 g. (85%) of VI, m.p. 207°. This material was identical with the azlactone VI obtained in reaction 1, since the two gave no depression in melting point.

Compound XII (5 g.) was heated with 50 cc. of acetic anhydride in exactly the same manner as VII above and was recrystallized from hot ethanol affording 3.5 g. (74%) of colorless XIII which is isomeric with the azlactone VI. After several crystallizations from ethanol it melted at 191°. Mixed with the azlactone VI it gave an appreciable depression in melting point.

Anal. Calc'd for $C_{18}H_{16}N_2O_3$: C, 70.13; H, 5.19; N, 9.09. Found: C, 69.88; H, 5.47; N, 9.32.

7. *Hydrolysis of the azlactones* VI *and* XIII. The experiments were carried out by refluxing weighted quantities of the azlactones VI and XIII in 100 cc. of water. After a definite time of boiling the mixture was cooled to room temperature, the precipitate was filtered, and was treated with 2% aqueous sodium bicarbonate. The alkaline solution again was filtered and the filtrate was acidified to Congo Red. The separated acid was collected, dried in air and in a vacuum desiccator over P_2O_5, and weighed.

No. of experiment	Azlactones VI m.p. 207° g.	Azlactones XIII m.p. 191° g.	Hours of heating	The hydrolyzed part acid VII m.p. 201° g.	The hydrolyzed part acid XII m.p. 238° g.	Per cent hydrolyzed VI	Per cent hydrolyzed XIII
1.	0.5026	0.5482	2	0.0014	0.0765	0.27	13.96
2.	0.5218	0.5372	4	0.0021	0.1065	0.40	19.83
3.	0.5127	0.5262	8	0.0115	0.1241	2.24	23.59
4.	0.5296	0.5731	24	0.0198	0.2756	3.74	48.09
5.	0.5317	0.5268	72	0.0356	0.4721	6.69	89.61

8. *Azlactones* V, XV *from α-benzoylamino-β-acetylamino-β-phenylpropionic acids* VII, XII. The acid VII (5 g.) was heated on the water-bath with 50 cc. of acetic anhydride for 1 hour and the anhydride was removed *in vacuo.* The residue was treated with ice-cold water and after it had solidified it was recrystallized from ethanol. The yellow crystalline substance was filtered, dried, dissolved in 50 cc. of ether and filtered again. The ether was removed from the ethereal solution and the residue was dissolved in ethanol. The separated yellow compound melted at 165–166° and was identical with V. The part insoluble in ether was recrystallized from ethanol and melted at 207°. It proved identical with VI.

Compound XII (5 g.) was treated in the same way as VII. From the ethanol solution a colorless substance (1.8 g.) was obtained and was recrystallized from the same solvent. It melted at 191° and was identical with XIII. By concentrating the ethanol filtrate a further amount (0.4 g.) of XIII was obtained. Total yield: 2.2 g. or 47%. The sirupy residue, after evaporation of the ethanol *in vacuo* was recrystallized from benzene. On longer standing in the cold, a deep yellow crystalline substance (XV) was obtained, melting at 140–142° (0.7 g. or 14%). Recrystallized from benzene it had a m.p. 143–145°.[15,16]

9. *Conversion of azlactone* XV *to azlactone* V. The azlactone XV (0.5 g.) was heated for 15 minutes with 10 cc. of 96% ethanol which contained 5–6 drops of pure pyridine and the clear solution was allowed to cool. From the intensely yellow ethanol filtrate, after longer standing in the cold, a yellow crystalline substance separated which, after recrystallization from benzene, had a m.p. 165–166° and corresponded to V, obtained in reactions 1 and 8.

10. *Anilide* (IX) *of α-benzoylamino-β-acetylamino-β-phenylpropionic acid.* The azlactone VI (3 g.) was heated for 2 hours at 190–200° (oil-bath) in a current of nitrogen with 300 cc. of freshly distilled aniline. The reaction mixture was cooled and the anilide was precipitated with 100 cc. of ether. The colorless substance was filtered off and dried. The yield of crude product was 3.5 g. or 90%. Recrystallization from 96% ethanol gave the pure anilide IX, m.p. 298–300°.

Anal. Calc'd for $C_{24}H_{23}N_3O_3$: C, 71.82; H, 5.73; N, 10.48. Found: C, 71.60; H, 5.72; N, 10.76.

The azlactone XIII (3 g.) was heated with 30 cc. of anilide and treated as described above. The yield of crude anilide was 3.2 g. or 82%, which by recrystallization from ethanol melted at 298–300° and proved to be identical with IX.

11. *Monohydrochloride* (X) *of α,β-diamino-β-phenylpropionic acid.* The azlactone VI (10 g.) was refluxed with 60 cc. of conc'd HCl for 2 hours. After the solution was cooled, it was extracted with 250 cc. of ether, the ethereal solution was washed with water until neutral, dried over sodium sulfate, and the ether was removed. The residue in the flask consisted of *benzoic acid* (1.8 g., 45%) and some benzaldehyde. By crystallization from water pure benzoic acid, m.p. 121–122°, was obtained. The acidic aqueous filtrate was evaporated *in vacuo* to dryness and the solid residue was dissolved in 100 cc. of 50% ethanol. On cooling, a colorless substance crystallized, which was insoluble in dry ether, ethyl acetate, benzene, and absolute ethanol, but readily soluble in water. Recrystallized from water, 4.1 g. (58%) of *monohydrochloride* X, m.p. 231°, was obtained.

Anal. Calc'd for $C_9H_{13}ClN_2O_2$: N, 12.93; Cl, 16.40. Found: N, 12.98; Cl, 16.27.

The azlactone XIII (5 g.) was hydrolyzed with 30 cc. of conc'd HCl. *Benzoic acid* (0.5 g., 25%) and 1.9 g. (54%) of the *monohydrochloride* X were obtained. Recrystallized from water it melted at 231° and showed no depression in melting point when mixed with the monohydrochloride obtained from VI.

The acids VII (5 g.) and XII (5 g.) were each hydrolyzed with 30 cc. of conc'd HCl, yielding 0.6 g. (32%) and 0.7 g. (37%) of *benzoic acid*, and 2.1 g. (63%) and 2 g. (60%) of *monohydrochloride* X, m.p. 231°, respectively.

12. *α,β-Diamino-β-phenylpropionic acid* (XI). The monohydrochloride X (5 g.) was dissolved in 80 cc. of water and silver oxide, prepared from 4.3 g. of silver nitrate in the usual way, was added to the solution. The mixture was allowed to stand overnight. The separated silver chloride was filtered off, washed with water, and the filtrate was saturated with hydrogen sulfide. The precipitate was removed by filtration, and the aqueous solution was boiled with charcoal for 15 minutes, filtered hot and evaporated *in vacuo* to dryness. The residue (3.3 g. or 79%) after several crystallizations from 50% ethanol, melted at 215–216°, and consisted of the free *α,β-diamino-β-phenylpropionic acid* (XI). The same acid was also obtained by neutralization of X, dissolved in water, with an equivalent amount of *N*/1 solution of sodium hydroxide. The acid XI is a colorless substance, showing a strong alkaline reaction in aqueous solution, insoluble in dry ether, benzene and absolute ethanol, readily soluble in hot, but sparingly in cold water.

Anal. Calc'd for $C_9H_{12}N_2O_2$: C, 60.00; H, 6.66; N, 15.55. Found: C, 60.11; H, 6.70; N, 15.88.

13. *α-Benzoylamino-β-acetylamino-β-(4-methylphenyl)propionic acid* (III, R = $C_6H_4CH_3$). To a solution of hippuric acid (7.2 g., $^1/_{25}$ mole) in 12 g. ($^1/_5$ mole) of glacial acetic acid and 4.1 g. ($^1/_{25}$ mole) of acetic anhydride, 8.8 ($^1/_{25}$ mole) of 4-methylbenzylidenebisacetamide was added at once, and the mixture was heated for 1 hour and 20 minutes. The reaction mixture which solidified on the addition of ice-cold water was dissolved in 70 cc. of 96% ethanol and afforded, on cooling, a yellow precipitate (1 g., 9.5%) of I (R = $C_6H_4CH_3$), m.p. 138–140°.[25] Recrystallization from ethanol raised the melting point of the azlactone to 141–142°. The same substance was obtained previously by the condensation of 4-methylbenzaldehyde with hippuric acid in acetic anhydride and anhydrous sodium acetate.[25] After the unsaturated azlactone was isolated and ethanol from the filtrate was removed *in vacuo*, the residue, which solidified upon the addition of ice-cold water, was filtered, dried, and treated with 50 cc. of chloroform. The insoluble part (2.8 g., 21%) was recrystallized from ethanol and gave the colorless III (R = $C_6H_4CH_3$), m.p. 229°. This substance is insoluble in water, ether, benzene and ethyl acetate, soluble in hot ethanol. It is easily soluble in sodium bicarbonate solution from which it can be precipitated with dilute mineral acids.

Anal. Calc'd for $C_{19}H_{20}N_2O_4$: C, 67.06; H, 5.88; N, 8.23. Found: C, 67.11; H, 6.03; N, 8.07.

The molecular weight was determined by titration in ethanol solution. Calc'd: 340. Found: 344.

After the acid, m.p. 229°, was separated, the chloroform was evaporated *in vacuo*, and the residue (7 g.), which solidified by the addition of water, was submitted to hydrolysis, either with water or 10% aqueous sodium hydroxide.

a. *Hydrolysis with water.* The crude product (2 g.) was heated with 100 cc. of water and then was treated as in 2, yielding 1.7 g. of isomeric acid III (R = $C_6H_4CH_3$), which upon crystallization from ethyl acetate melted at 197°. This compound is readily soluble in ethanol, insoluble in ether and water, sparingly soluble in benzene and ethyl acetate.

Anal. Calc'd for $C_{19}H_{20}N_2O_4$: C, 67.06; H, 5.88; N, 8.23. Found: C, 67.02; H, 6.20; N, 8.22.

The molecular weight was determined by titration in ethanol solution. Calc'd: 340. Found: 343.

b. *Hydrolysis with hydroxide solution.* The remaining crude product (5 g.) was dissolved in 25 cc. of 96% ethanol, 40 cc. of 10% aqueous sodium hydroxide was added, and the mixture was allowed to stand for 24 hours at room temperature. Upon acidifying the solution with 10% hydrochloric acid (to Congo Red), a yellowish substance separated, which was filtered, dried and then washed with a little cold ethyl acetate. It had a melting point of 193–195°, and its yield was 2.9 g., making the total yield of hydrolyzed compound 4.6 g. (34%). By recrystallization from ethyl acetate the m.p. of the acid was raised to 197°, making it identical with that obtained under a.

14. *2-Phenyl-4-(4-methylbenzylidene)-5-oxazolone,* (I, R = $C_6H_4CH_3$). The acid III (R = $C_6H_4CH_3$), m.p. 229°, (2 g.) was heated with 20 cc. of acetic anhydride on the waterbath for 15 minutes. The anhydride was removed *in vacuo* and the residue was dissolved in 96% ethanol. On cooling, yellow crystals were deposited, melting at 140–141°, showing no depression in melting point with the azlactone obtained under 13. The yield was 1.3 g. or 84%.

The acid III (R = $C_6H_4CH_3$) m.p. 197° (2 g.) was treated in exactly the same manner as above. The yield of azlactone I (R = $C_6H_4CH_3$), m.p. 140–141°, was 1.2 g. or 78%.

15. *α-Benzoylamino-β-acetylamino-β-(3,4-methylenedioxyphenyl)propionic acid* (III, R = $C_6H_3O_2CH_2$). Hippuric acid (7.2 g., $^1/_{25}$ mole) was dissolved in 12 g. ($^1/_5$ mole) of glacial acetic acid and 4.1 g. ($^1/_{25}$ mole) of acetic anhydride at 110°. 3,4-Methylenedioxybenzylidenebisacetamide (10 g., $^1/_{25}$ mole) then was introduced, and after it had dissolved the mixture was heated for 80 minutes. Further treatment was the same as that described under 1. There was obtained 11.5 g. of crude product which was dissolved in 100 cc. of 96% ethanol. On cooling, the azlactone I (R = $C_6H_3O_2CH_2$), m.p. 194–195° (0.6 g. or 5%) was collected. Recrystallization from ethanol raised the m.p. to 197°. This compound had been obtained by many authors by condensing 3,4-methylenedioxybenzaldehyde with hippuric acid in acetic anhydride and anhydrous sodium acetate.[26] After the isolation of the unsaturated azlactone (m.p. 197°), the ethanol filtrate was concentrated to 50 cc. and allowed to stand in the cold. After several days the separated crystalline substance was filtered and washed with a little cold ethanol. The yield was 1 g. of the colorless compound, melting at 228–230°. It is soluble in ether, benzene, ethyl acetate and water, soluble in hot ethanol. It is easily soluble in sodium bicarbonate solution from which it is precipitated with dilute mineral acids. Recrystallized from ethanol it had a melting point of 232°, and was identified as III (R = $C_6H_3O_2CH_2$).

Anal. Calc'd for $C_{19}H_{18}N_2O_6$: C, 61.62; H, 4.86; N, 7.56. Found: C, 61.76; H, 4.66; N, 7.74.

The molecular weight was determined by titration in ethanol solution. Calc'd: 370. Found: 372.

After the acid, m.p. 232°, was removed, the ethanol from the filtrate was evaporated and the solid residue was treated with chloroform. The insoluble part (3.2 g., 22%), m.p. 216–218°, was recrystallized from ethanol, giving a colorless substance melting at 220°. It is insoluble in water, sparingly soluble in ethyl acetate and benzene, readily soluble in hot ethanol. It dissolves easily in sodium bicarbonate solution and is reprecipitated on acidification. According to analysis this substance represents a second isomeric III (R = $C_6H_3O_2CH_2$).

Anal. Calc'd for $C_{19}H_{18}N_2O_6$: C, 61.62; H, 4.86; N, 7.56. Found: C, 61.61; H, 4.69; N, 7.67.

The molecular weight determined by titration was 373. Calc'd: 370.

The remaining chloroform solution was evaporated to dryness and the solid residue (5 g.) was saponified with aqueous NaOH as under 13 b. A colorless substance, m.p. 227–230°, was obtained (3.2 g.) which, recrystallized from ethanol, melted at 232°. It proved identical with the first isomeric III (R = $C_6H_3O_2CH_2$), increasing, thus, the total yield to 4.2 g. (28%).

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(25) Dakin, *J. Biol. Chem.*, **9**, 151 (1911).

(26) Kropp and Decker, *Ber.*, **42**, 1184 (1909).

16. *2-Phenyl-4-(3,4-methylenedioxybenzylidene)-5-oxazolone* (I, R = $C_6H_3O_2CH_2$). The acid III (R = $C_6H_3O_2CH_2$), m.p. 232°, (2 g.) was heated with 20 cc. of acetic anhydride on the water bath for 15 minutes and the anhydride was removed *in vacuo*. The residue was treated with ice-cold water, and after it had solidified, was recrystallized from ethanol, yielding 1.2 g. (76%) of I (R = $C_6H_3O_2CH_2$), which, after another crystallization from ethanol, melted at 197°. A mixture with a sample obtained in reaction 15 showed no depression in melting point.

The acid III (R = $C_6H_3O_2CH_2$), m.p. 220°, (2 g.), when treated in the same way as the isomeric acid, m.p. 232°, gave 1 g. (63%) of I (R = $C_6H_3O_2CH_2$), melting at 195–196°.

17. *Azlactone* (II, R = $C_6H_4OCH_3$) *of α-benzoylamino-β-acetylamino-β-(2-methoxyphenyl)-propionic acid, and α-benzoylamino-β-acetylamino-β-(2-methoxyphenyl)-propionic acid* (III, R = $C_6H_4OCH_3$). Hippuric acid (7.2 g., $^1/_{25}$ mole) was dissolved in a mixture of 12 g. ($^1/_5$ mole) of glacial acetic acid and 4.1 g. ($^1/_{25}$ mole) of acetic anhydride heated at 110°, and to the clear solution 9.4 g. ($^1/_{25}$ mole) of 2-methoxybenzylidenebisacetamide was added and the mixture was heated for 75 minutes. Further treatment as described under 1, afforded 12.5 g. of a yellowish product. Dissolved in 250 cc. of 96% ethanol it deposited, on cooling, a colorless crystalline substance (4.5 g.), which was collected by filtration and washed with a little cold ethanol. This product, melting at 237–239°, was insoluble in ether, benzene, ethyl acetate and water, and soluble in hot ethanol and acetone. Recrystallized from ethanol it melted at 241°. It dissolved easily in aqueous sodium bicarbonate and could be precipitated by acidification. The analysis corresponded to the acid III (R = $C_6H_4OCH_3$).

Anal. Calc'd for $C_{19}H_{20}N_2O_5$: C, 64.04; H, 5.62; N, 7.86. Found: C, 63.93; H, 5.85; N, 7.86.

The molecular weight was determined by titration. Found: 357. Calc'd: 356.

After the acid (m.p. 241°) was removed, the filtrate was concentrated to 150–170 cc. and, on cooling, deposited a yellow substance which was filtered off and washed with a little cold ethanol. This product (0.7 g. or 6%) proved to be the unsaturated azlactone I (R = $C_6H_4OCH_3$), melting at 154–156°, which was also obtained by condensing 2-methoxybenzaldehyde with hippuric acid in the presence of acetic anhydride and anhydrous sodium acetate.[27] After the removal of I (R = $C_6H_4OCH_3$), the filtrate was further concentrated to 40–50 cc. and allowed to stand in the cold for several days. The separated saturated azlactone II (R = $C_6H_4OCH_3$) (1.9 g., 14%) was filtered off and washed with ether. It melted at 180° and was almost colorless. Recrystallization from ethanol raised the m.p. to 185°. The azlactone is insoluble in water, ether and ethyl acetate, sparingly soluble in benzene, readily soluble in hot ethanol. It was not dissolved by diluted alkali.

Anal. Calc'd for $C_{19}H_{18}N_2O_4$: C, 67.45; H, 5.32; N, 8.28. Found: C, 67.25; H, 5.24; N, 8.53.

The ethanol filtrate, after the azlactone (m.p. 185°) was removed, was left in the cold for 2 weeks. The separated crystalline substance, m.p. 150–153° (1.7 g. or 12%) was treated with 50 cc. of a 3% solution of ammonium hydroxide and was shaken vigorously. The mixture was filtered, the filtrate acidified with dilute hydrochloric acid (to Congo Red) and the separated acid was filtered off and dried. The yield was 1.6 g. or 11% of the colorless isomeric acid III (R = $C_6H_4OCH_3$), melting at 141–142°, which after crystallization from ethanol melted at 145°. It is insoluble in ether and benzene, sparingly soluble in ethyl acetate, readily soluble in hot ethanol.

Anal. Calc'd for $C_{19}H_{20}N_2O_5$: C, 64.04; H, 5.62; N, 7.86. Found: C, 63.95; H, 5.84; N, 7.96.

The molecular weight determined by titration was 360. Calc'd: 356.

After the acid (m.p. 145°) was removed, the alcohol was evaporated from the filtrate, and the solid residue was (3.5 g.) hydrolyzed with 10% aqueous sodium hydroxide at room temperature. By acidification with 10% hydrochloric acid (to Congo Red) an acid was obtained, which was collected, dried, and then washed with ethyl acetate, giving 2 g. of a product melting at 237–239°. Recrystallization from ethanol raised the m.p. to 241°. This substance, mixed with the acid, m.p. 241°, obtained as the first product in this reaction, showed no depression in melting point. The total yield of the acid III (R = $C_6H_4OCH_3$), was therefore 6.5 g. or 46%.

18. *2-Phenyl-4-(2-methoxybenzylidene)-5-oxazolone* (I, R = $C_6H_4OCH_3$). The acid III (R = $C_6H_4OCH_3$), m.p. 241°, (1 g.) was heated with 10 cc. of acetic anhydride for 1 hour on the water bath, giving 0.5 g. (64%) of a yellow crystalline substance, m.p. 152–153°, which, recrystallized from ethanol, melted at 154–156°, and showed no depression in melting point when mixed with I (R = $C_6H_4OCH_3$).

The isomeric acid, m.p. 145°, (1 g.) treated in the same manner as above gave 0.5 g. (64%) of I (R = $C_6H_4OCH_3$), m.p. 150–153° (recrystallized from ethanol it melted at 154–156°). In addition, a second product was also obtained which proved to be the azlactone II (R = $C_6H_4OCH_3$), m.p. 185°, insoluble in ether and identical with the saturated azlactone formed in reaction 17. The yield was 0.2 g. or 21%.

19. *Azlactone* (II, R = $C_6H_4OCH_3$) *of α-benzoylamino-β-acetylamino-β-(2-methoxyphenyl)propionic acid.* The acid III (R = $C_6H_4OCH_3$), m.p. 145°, (1 g.) was heated with 10 cc. of acetic anhydride for 10 minutes, yielding 0.6 g. (63%) of II (R = $C_6H_4OCH_3$), melting at 180–181°. Recrystallization from ethanol raised the melting point to 185°. It showed no depression in melting point when mixed with the saturated azlactone obtained in reaction 17.

From the azlactone II (R = $C_6H_4OCH_3$), the starting acid III (R = $C_6H_4OCH_3$), m.p. 145°, was readily obtained by hydrolysis in a yield of 76%.

20. *α-Benzoylamino-β-acetylamino-β-(4-methoxyphenyl)-propionic acid* (III, R = $C_6H_4OCH_3$). Hippuric acid (7.2 g., $^1/_{25}$ mole) was dissolved at 115° in 12 g. ($^1/_5$ mole) of glacial acetic acid and 4.1 g. ($^1/_{25}$ mole) of acetic anhydride, and to the clear solution 9.4 g. ($^1/_{25}$ mole) of 4-methoxybenzylidenebisacetamide was added, and heating was continued for 1 hour and 10 minutes. The reaction mixture then was treated as in 1, affording 12 g. of a crude yellowish product which was dissolved in 200 cc. of 96% ethanol. On cooling to room temperature a yellow crystalline substance separated, melting at 150–153°, which was recrystallized from the same solvent and gave 1.2 g. (11%) of the azlactone I (R = $C_6H_4OCH_3$), m.p. 157–158°, obtained also by condensing 4-methoxybenzaldehyde with hippuric acid in acetic anhydride and anhydrous sodium acetate.[12]

After the unsaturated azlactone was removed, the ethanol filtrate was allowed to stand in the cold for 48 hours. The separated crystals were filtered off and washed with a little cold ethanol, giving a colorless substance, m.p. 227–229°, which was insoluble in water, benzene and ethyl acetate, and soluble in hot ethanol. After recrystallization from ethanol the melting point was raised to 232°. The substance was readily soluble in dilute sodium bicarbonate solution and was reprecipitated by acidification with dilute acids. The analysis corresponded to the acid III (R = $C_6H_4OCH_3$). The yield of the crude product (m.p. 228–230°) was 6.2 g. or 44%.

Anal. Calc'd for $C_{19}H_{20}N_2O_5$: C, 64.04; H, 5.62; N, 7.86. Found: C, 64.12; H, 5.62; N, 7.92.

The molecular weight determined by titration was 357. Calc'd: 356.

After the acid, m.p. 232°, was isolated, the volume of the alcoholic filtrate was reduced to about 50–70 cc., and the solution was allowed to stand in the cold. After two weeks,

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(27) Bergel, Haworth, Morrison, and Rinderknecht, *J. Chem. Soc.*, 261 (1944).

the precipitate was collected by filtration and washed with ethanol. It melted at 210–214°, and was readily soluble in acetone, and insoluble in benzene, ether and water. It dissolved in sodium bicarbonate solution and was reprecipitated on acidifying with mineral acids. After recrystallization from 80% ethanol it melted at 218°. The analysis corresponded to the second isomeric acid III (R = $C_6H_4OCH_3$). The yield was 2.1 g. or 15%.

Anal. Calc'd for $C_{19}H_{20}N_2O_5$: C, 64.04; H, 5.62; N, 7.86. Found: C, 63.92; H, 5.74; N, 7.91.

The molecular weight determined by titration was 359. Calc'd: 356.

21. *2-Phenyl-4-(4-methoxybenzylidene)-5-oxazolone* (I, R = $C_6H_4OCH_3$). The acid III (R = $C_6H_4OCH_3$), m.p. 232°, (2 g.) was heated for 15 minutes with 20 cc. of acetic anhydride, affording 1.3 g. (83%) of the azlactone I (R = $C_6H_4OCH_3$), m.p. 151–153°. Recrystallized from ethanol it melted at 157–158°, and showed no depression in melting point when mixed with the oxazolone obtained in reaction 20.

The acid III (R = $C_6H_4OCH_3$), m.p. 218°, (2 g.) treated in the same manner gave 1.2 g. (77%) of the azlactone I (R = $C_6H_4OCH_3$), m.p. 152–154°, which recrystallized from ethanol melted at 157–158°.

22. *2-Phenyl-4-(3-nitrobenzylidene)-5-oxazolone* (I, R = $C_6H_4NO_2$) and *α-benzoylamino-β-(3-nitrophenyl)acrylic acid.* Hippuric acid (7.2 g., $^1/_{25}$ mole) was dissolved at 110° in 30 g. ($^1/_2$ mole) of glacial acetic acid and 4.1 g. ($^1/_{25}$ mole) of acetic anhydride; to this solution 10 g. ($^1/_{25}$ mole) of 3-nitrobenzylidenebisacetamide was added and after it had dissolved, at 140°, heating was continued for another 2 hours at 125–130°. The mixture was cooled and poured in cold water. The solid mass was filtered off and treated with a 3% aqueous solution of sodium bicarbonate. The solution was filtered from the insoluble part, acidified with 10% HCl (to Congo Red), and the separated substance was filtered, dried, and washed with ethyl acetate, giving 6 g. (48%) of *α-benzoylamino-β-(3-nitrophenyl)acrylic acid.* Recrystallized from ethanol it melted at 218–220°.[19]

Anal. Calc'd for $C_{16}H_{12}N_2O_5$: C, 61.54; H, 3.84; N, 8.97. Found: C, 61.89; H, 4.15; N, 9.20.

The part insoluble in 3% sodium bicarbonate solution was washed with water and dried. The crude product melted at 167–170°, and after recrystallization from ethanol gave the pure azlactone of *α-benzoylamino-β-(3-nitrophenyl)-acrylic acid,* m.p. 174° (I, R = $C_6H_4NO_2$).[19] The yield was 2 g. or 17%.

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