Schrecker of this Laboratory and L. V. Heisey of Bridgewater College for helpful discussions during the preparation of this manuscript.
BETHESDA 14, MD.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, AMHERST COLLEGE]

The Synthesis of Analogs of Penicillin.[1a] I

BY DAVID TODD[1b] AND SYLVIA TEICH

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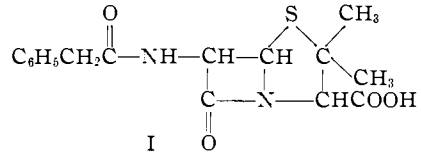
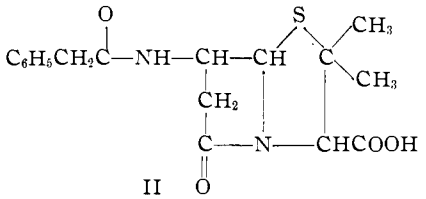
The condensation of L-cysteine and of DL-penicillamine with methyl γ-formylbutyrate gives the bicyclic thiazolidine lactams Va and Vb, whose structure is proved *via* nickel desulfurization. An attempt to prepare the vinylog IV of penicillin is described.

The chemical mechanism of the antibiotic action of penicillin (I) remains obscure in spite of the fact that much biological investigation[2] tends to show that penicillin may compete with glutathione in processes involving hydrogen transfer from –SH groups on molecules involved in the respiratory systems of many organisms. Because of the well-known instability of the β-lactam ring[3] in penicillin, it is not unreasonable to suppose that at some point in the metabolism of penicillin the β-lactam ring is opened. It is, therefore, of interest to modify this particular structural feature of penicillin in order to see if an easily-opened β-lactam ring is a *sine qua non* for its antibiotic action.

Two modifications of the β-lactam ring have already been reported, and in each case the resulting compound was found to be devoid of antibiotic action. du Vigneaud and Carpenter[4] have prepared the γ-lactam II, and Neher and co-workers[5] have prepared acylated thiazolidines of the type III in which the β-lactam ring has been formally hydrogenated open between the two asymmetric carbon atoms.



I



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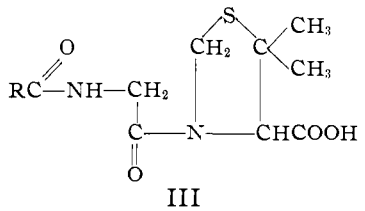
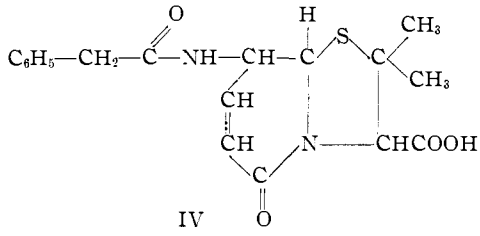
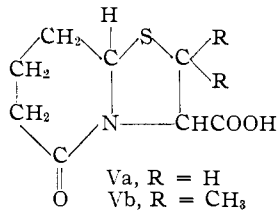


III

It seemed to us of interest to prepare compounds in which the β-lactam ring of penicillin is enlarged to a six-membered ring, and in particular the vinylog (IV) of penicillin. In this paper we report the synthesis of two δ-lactams, Va and Vb, and an attempt to prepare IV.

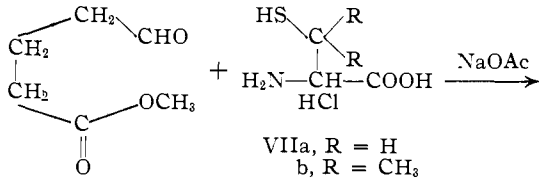


IV



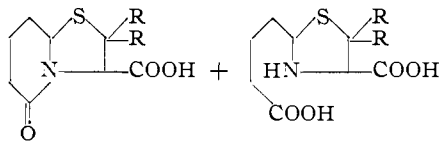
Va, R = H
Vb, R = CH_3

Methyl γ-formylbutyrate (VI) was condensed with L-cysteine hydrochloride (VIIIa) in an aqueous alcoholic solution containing sodium acetate or sodium hydroxide to give the bicyclic thiazolidine (Va) in 25–35% yield.



VI

VIIa, R = H
b, R = CH_3



Va, R = H
b, R = CH_3

VIII

The presumed intermediate amine-ester was never isolated, the cyclization to V occurring readily under mild conditions. However, under certain conditions, a second compound was isolated whose analysis corresponded to that of VIII. Since it could not be readily cyclized to Va it was not further characterized. Although attempts to convert Va

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(1) (a) This work was supported by the Office of Naval Research and by the Research Corporation. (b) Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

(2) R. Pratt and J. Dufrenoy, *Bact. Rev.*, **12**, 79 (1948).

(3) R. Holley and A. Holley, THIS JOURNAL, **72**, 2771 (1950).

(4) V. du Vigneaud and F. Carpenter, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, Ch. 27.

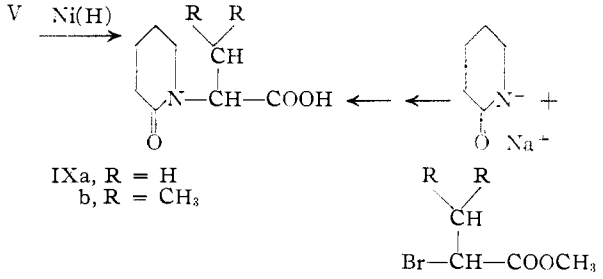
(5) R. Neher, *et al.*, *Helv. Chim. Acta*, **29**, 1815 1874 (1946).

to the methyl ester gave no solid product, a crystalline sulfone was obtained.

Condensation of the acetone derivative of cysteine, 2,2-dimethyl-4-thiazolidinecarboxylic acid, with VI likewise led to the formation of Va.

Reductive desulfurization of the sodium salt of Va in aqueous solution by means of Raney nickel afforded optically active α-1-(2-piperidone)-propionic acid (IXa) in 30% yield.[6]

The DL-form of this compound was synthesized by condensation of the sodium salt of 2-piperidone with methyl α-bromopropionate, followed by hydrolysis of the ester to the acid, which though optically inactive melted only four degrees below the compound obtained by degradation. Comparison of the infrared spectra of these compounds shows them to be structurally identical.

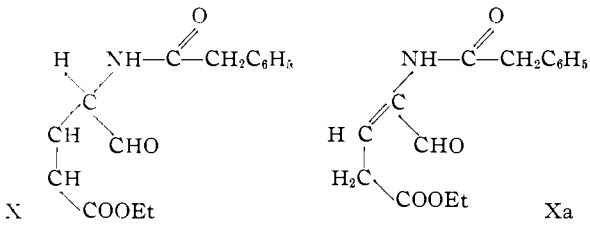


Although it is theoretically possible[7] for the O-alkylated product to be formed from 2-piperidone, the evidence indicates that the product is actually the N-alkylated one, a result in harmony with previous findings in analogous cases.[8] Hydrolysis of IXa with concentrated hydrochloric acid followed by recyclization through the N-benzoyl derivative by the method of Schotten[9] gave IXa back again. An O-alkyl side chain would be cleaved under the acid conditions of the hydrolysis.

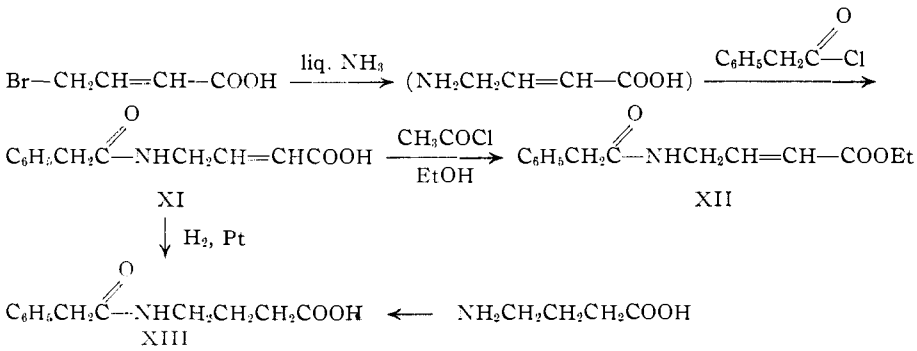
The synthesis of Vb was then undertaken, making use of the aldehyde-ester VI and DL-penicillamine hydrochloride. The desired product was obtained in 59% yield, and was desulfurized as described above to give IXb. This was synthesized in the previously described manner from the sodium salt of 2-piperidone and ethyl α-bromoisovalerate. The two products so obtained were found to be identical.

Since attempts to introduce the double bond into the desired position of Va failed, and preliminary experiments on the γ-bromination of N-crotonylpiperidine and of N,N-diphenylcrotonamide produced mostly tars, the approach to the synthesis of the vinylog IV *via* Vb was abandoned.

Our attention turned to the possibility of employing the appropriately substituted α,β-unsaturated aldehyde in the condensation with cysteine (or penicillamine). Although the synthesis of thiazolidines from saturated aldehydes or ketones has received considerable attention,[10] relatively little work has been done on the reaction with α,β-unsaturated carbonyl compounds. The failure of various α,β-unsaturated ketosteroids to yield thiazolidines has been reported[11] though crystalline products were obtained with some simpler compounds. In addition, the fact that the diethyl mercaptal of crotonaldehyde can be prepared in good yield under conditions which differ considerably from those required for the addition of ethyl mercaptan across the double bond of crotonaldehyde[12] made it seem possible that preparation of the vinylog IV in the usual manner by the condensation of γ-formyl-γ-phenylacetylaminocrotonic ester, X, with penicillamine could be carried out, it being borne in mind that the glutaconic half aldehyde system of X might be capable of isomerism to the structure Xa.



The proposed synthesis of X involved the γ-formylation of ethyl γ-phenylacetylaminocrotonate (XII) by ethyl formate. A preliminary attempt to prepare XII by the Gabriel method failed; although ethyl γ-phthalimidocrotonate was readily prepared in the normal manner, acid hydrolysis gave no identifiable product. The successful route to XII is



γ-Bromocrotonic acid was aminated successfully with liquid ammonia and the intermediate amino acid phenylacetylated directly by the Schotten–Baumann method to give XI. The usual methods of esterification gave poor results when applied to XI. Thus Fischer esterification with hydrochloric acid gave the saturated ester, ethyl β-chloro-

(6) See ref. 5, p. 1015, for a similar retention of optical activity.

(7) G. Wheland, "Advanced Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., pp. 617–621.

(8) L. Ruzicka, *Helv. Chim. Acta*, **4**, 472 (1921); J. Tafel and O. Wassmuth, *Ber.*, **40**, 2831 (1907).

(9) C. Schotten, *ibid.*, **21**, 2235 (1888).

(10) M. Schubert, *J. Biol. Chem.*, **111**, 671 (1935); **114**, 341 (1936); **121**, 539 (1937); S. Ratner and H. Clarke, THIS JOURNAL, **59**, 200 (1937). See also "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., Ch. 25.

(11) S. Liebermann, P. Brazeau and L. Hariton, THIS JOURNAL, **70**, 3094 (1948).

(12) R. Hall and B. Howe, *J. Chem. Soc.*, 2723 (1949).

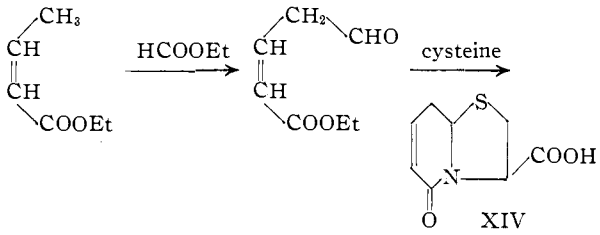
butyrate, a type of addition that is well-known.[13] The treatment of XI with acetyl chloride and ethanol at room temperature afforded the desired ester, XII, in 83% yield.

Since there is a possibility that an allylic rearrangement might have occurred during the amination of the γ-bromo acid, the structure of XI was proved by hydrogenation to γ-phenylacetylaminobutyric acid XIII, a sample of which was prepared in the usual manner from ν-aminobutyric acid. The two products proved to be identical. Furthermore, α-phenylacetylaminobutyric acid was prepared and found to be quite different from the amination product.

Attempts to introduce a γ-formyl group into XII *via* ethyl formate in the presence of basic catalysts have failed to give the desired product, X. Similar attempts to formylate ethyl crotonate, as described in the literature[14] served to confirm the later report[15] that the γ-formyl compound is only formed in very low yield.

We attempted to condense the crude formylated ethyl crotonate with cysteine, but none of the desired condensation product XIV could be isolated.

An alternative route of X is indicated in the work of Butenandt[16] who was able to introduce a diethylacetal group into ethyl chloroacetate. However, we have found that ethyl formate will not condense with ethyl γ-bromocrotonate in the presence of potassium ethoxide under Butenandt's conditions. The evidence then indicates that as far as Claisen condensations are concerned the γ-carbon hydrogens of substituted crotonic esters are considerably less reactive than the hydrogens of the corresponding acetates.



In anticipation of the necessity of introducing a γ-phenylacetylamino group in XIV some experiments were carried out on the γ-bromination of N,N-disubstituted crotonamides. N,N-Diphenylcrotonamide and N-crotonylpiperidine were prepared and treated with N-bromosuccinimide, but no products could be isolated. This would indicate there is little likelihood of being able to introduce a bromine atom into the γ-position of XIV.

However, it was found possible to prepare γ-bromocrotonaldehyde diethylacetal (prepared in 82% yield by the method of Fischer and Baer[17]) though the material suffered considerable decomposition on vacuum distillation. It was characterized by conversion to the dinitrophenylhydrazone.

(13) E. Erdmann and F. Bedford, *Ber.*, **42**, 1327 (1909); Moureau, *et al.*, *Compt. rend.*, **172**, 1267 (1921).

(14) W. Borsche and R. Manteuffel, *Ann.*, **505**, 177 (1933).

(15) R. Elderfield and J. Fried, *J. Org. Chem.*, **6**, 566 (1941).

(16) A. Butenandt, H. Jatzkewitz and U. Schiedt, *Z. phyiol. Chem.*, **283**, 209 (1948).

(17) H. Fischer and E. Baer, *Helv. Chim. Acta*, **18**, 514 (1935).

Experimental[18]

Methyl γ-formylbutyrate was prepared by the previously described method through the Rosenmund reduction of γ-carbomethoxybutyryl chloride,[19] in the presence of the Rosenmund poison. The yields were erratic and low in spite of the evolution of 70–85% of the calculated amount of HCl during the reduction. The 2,4-dinitrophenylhydrazone was prepared and found to melt at 104–105° (reported[19b] 105–106°).

Condensation of L-Cysteine Hydrochloride with Methyl γ-Formylbutyrate. Method (a).[20]—A solution of 3.15 g. (0.020 mole) of L-cysteine hydrochloride and 1.68 g. (0.020 mole) of anhydrous sodium acetate in 15 cc. of water was added to a solution of 2.60 g. (0.020 mole) of methyl γ-formylbutyrate in 10 cc. of ethanol then 15 cc. more ethanol was added to clear the solution. After standing overnight at room temperature the solution was well cooled, and the separated solid filtered to give 0.70 g. (18%) of material which melted at 204–207°. It was quite soluble in 5% sodium bicarbonate, but showed no increased solubility in dilute hydrochloric acid. It gave a negative nitroprusside test for a free sulfhydryl group in sodium carbonate. After several crystallizations from water the material Va is obtained as bunches of rods, m.p. 212.5–214.0°.

Anal. Calcd. for $C_8H_{11}O_3NS$: C, 47.75; H, 5.51; N, 6.96; neut. equiv., 201. Found: C, 47.97, 47.62; H, 5.23, 5.38; N, 7.11; neut. equiv., 202; $[\alpha]^{26}D$ −223 ± 7° (0.64% in ethanol).

The sulfone was prepared by treatment of the compound with two equivalents of hydrogen peroxide in glacial acetic acid and allowing the solution to stand for two weeks at room temperature. Evaporation of the acetic acid and crystallization from ethanol gave a compound which melted at 203°. A mixture of this with the starting material exhibited a marked melting point depression. This material gave a negative permanganate test.

Anal. Calcd. for $C_8H_{11}O_5NS$: C, 41.19; H, 4.76. Found: C, 41.13; H, 4.91.

Method (b).—A mixture of 1.8 g. (0.014 mole) of methyl γ-formylbutyrate and 2.18 g. (0.014 mole) of L-cysteine hydrochloride in 50 cc. of ethanol was heated on the steam-bath for five minutes, and to the clarified solution 15.7 cc. of 0.10 *N* NaOH was added. The solution was refluxed ten minutes, concentrated *in vacuo* to about 25 cc. and well cooled. There was obtained 0.96 g. (39%) of shiny crystals, m.p. 190–195°. Two recrystallizations from ethanol yielded pure material, m.p. 213–214°, identical (mixed m.p.) with the material prepared in the alternative manner.

The filtrate from the above material was taken dry *in vacuo* to leave a viscous yellow residue. Extraction with ethanol gave 1.29 g. of a water-soluble ether-insoluble material, m.p. 165–175°, which gave a negative nitroprusside test for -SH and contained no halogen. Recrystallization from ethanol gave a product of m.p. 182.5–184.5°.

Anal. Calcd. for $C_8H_{13}O_4SN$: C, 43.82; H, 5.97. Found: C, 43.69; H, 5.65.

This material is presumably the diacid VIII corresponding to the lactam-acid Va.

Method (c).—The thiazolidinelactam may also be prepared in 48% crude yield (m.p. 204–211°) by allowing the aldehyde to condense with 2,2-dimethyl-4-thiazolidinecarboxylic acid hydrochloride in the presence of one equivalent of sodium acetate in aqueous methanol.

Reductive Desulfurization of the Lactam-Acid Va.—The method used was that previously applied to other analogs of penicillin.[21]

A solution of 0.80 g. (0.0040 mole) of the lactam Va in 80 cc. of 0.050 *N* NaOH was treated with 9 g. of *freshly prepared* Raney nickel[22] and the mixture placed in a bath at 170° for 20 minutes after the onset of boiling. The hot mixture was filtered, the catalyst washed well with water, the filtrate neutralized with hydrochloric acid and evaporated to small volume *in vacuo*. It was then subjected to continuous extraction with ethyl acetate. Concentration of

(18) All melting points are corrected.

(19) (a) S. Harris, *et al.*, THIS JOURNAL, **67**, 2096 (1945); (b) P. Clutterbuck and H. Raper, *Biochem. J.*, **19**, 385 (1925).

(20) Reference 5, Ch. 25.

(21) Reference 5, p. 1014.

(22) R. Mozingo, *et al.*, THIS JOURNAL, **65**, 1013 (1943).

the ethyl acetate extract and addition of hexane led to the precipitation of 0.20 g. (30%) of product, m.p. 143–146°. Several crystallizations from benzene–hexane gave material of m.p. 151.0–152.0° (sinters at 150°).

Anal. Calcd. for $C_8H_{13}NO_3$: C, 56.12; H, 7.65; N, 8.18. Found: C, 56.20; H, 7.61; N, 8.20; $[\alpha]^{20}D$ −53° (2.7% in EtOH); $[\alpha]^{23}D$ −42.6° (2.7% in EtOH) on another sample.

2-Piperidone was prepared by the Beckmann rearrangement of cyclopentanone oxime through the use of sulfuric acid.[23] The product had a boiling point of 135° at 15 mm.

α-1-(2-Piperidone)-propionic Acid.—To a stirred solution of 13.0 g. (0.13 mole) of 2-piperidone in 150 cc. of dry benzene was added 3.2 g. (0.13 mole) of finely divided sodium at room temperature. After one hour the solution had become colored slightly and a gel had separated. Dry benzene (100 cc.) was added and the mixture refluxed five hours with stirring. The gelatinous mass was cooled to 10° and a solution of 22 g. (0.13 mole) of methyl α-bromopropionate in 15 cc. of dry benzene was added over 15 minutes. Stirring was continued at room temperature for 14 hours. The reaction mixture was filtered, the solid precipitate washed well with benzene and ether, and the filtrate concentrated *in vacuo* at 100°. The residual oil was distilled *in vacuo* to give 12.0 g. (50%) of methyl α-1-(2-piperidone)-propionate, b.p. 156–157° (16 mm.); $n^{30}D$ 1.4715.

A solution of 7.1 g. (0.038 mole) of the ester and 2.2 g. (0.038 mole) of potassium hydroxide in about 25 cc. of dry methanol was left at room temperature for one day. The solution was saturated with carbon dioxide, the small precipitate of potassium carbonate filtered, and the methanol removed *in vacuo*. The resulting solid was treated with a little water and extracted with ether. Evaporation of the ethereal extracts gave 2.0 g. (28%) of starting material.

The aqueous solution was acidified with dilute acid, taken dry *in vacuo*, and the solid residue extracted with boiling ethyl acetate. Concentration of the ethyl acetate gave 4.9 g. (75%) of the desired acid, m.p. 140–143°. Recrystallization from ethyl acetate–hexane and benzene–hexane yielded a product melting constantly at 147.0–148.0° (softens at 145°). A mixture of this material and that produced in the reductive desulfurization reaction (m.p. 151–152°) melted at 147.0–150.0°. The infrared spectra, determined in 5% chloroform solution, of the DL- and L-compounds were found to be identical. Aside from the expected carbonyl and amide bands at 5.81 and 6.19 mμ, respectively, there is characteristic absorption at 6.66 mμ.

Anal. Calcd. for $C_8H_{13}NO_3$: C, 56.12; H, 7.65; N, 8.18; neut. equiv., 171. Found: C, 56.34; H, 7.43; N, 7.92; neut. equiv., 172.

When the hydrolysis of the ester was carried out in hot aqueous methanolic potassium hydroxide (one equivalent) for two hours, an 84% yield of crude product, m.p. 107–122°, was obtained. Several crystallizations from benzene–hexane and ethyl acetate raised the melting point to 145–147°.

Hydrolysis and Resynthesis of α-1-(2-Piperidone)-propionic Acid—A solution of 0.50 g. of the acid (synthesized from 2-piperidone) was heated at 100° in concentrated hydrochloric acid for one day, then taken dry *in vacuo*. The residue was dissolved in hot absolute ethanol, and dry ether added. A small amount of inorganic precipitate was filtered and the filtrate evaporated dry to give an oil that could not be induced to crystallize. The material (soluble in water) was washed well with ether and ethyl acetate to remove any starting material. It gave a precipitate with silver nitrate.

The crude hydrochloride was benzoylated in the usual way, barium hydroxide being used as the base. The barium salt was decomposed with excess 2 *N* sulfuric acid, the barium sulfate filtered, and the filtrate extracted with ether to remove benzoic acid. The aqueous solution was concentrated *in vacuo*, and extracted with ethyl acetate to remove any cyclized material. The aqueous solution was taken dry *in vacuo*, treated with 3 cc. of quinoline and heated at 180° in an oil-bath for five minutes. The reaction mixture was treated with dilute sodium bicarbonate and washed with ether. The basic solution was acidified, evaporated dry and the residue extracted with hot ethyl acetate. Concentration and addition of hexane gave 0.20 g. of colorless crystals, m.p. 144–145°. This material does not depress the melting point of the starting material.

Condensation of DL-Penicillamine with Methyl γ-Formylbutyrate.—Method (a) was used. When the reaction mixture was allowd to stand at room temperature for several months, 59% of stout prisms of the thiazolidinelactam (Vb) was obtained. After several crystallizations from water the compound has m.p. 157.0–158.5°.

Anal. Calcd. for $C_{10}H_{15}NO_3S$: C, 52.37; H, 6.60; N, 6.11. Found: C, 52.57; H, 6.56; N, 5.84.

The sulfone was prepared by adding 0.5 cc. of 30% hydrogen peroxide to a solution of 147 mg. of the thiazolidinelactam in 5 cc. of acetic acid. After standing at room temperature several days the solution was taken dry and the product crystallized several times from water. The sulfone was obtained as stout prisms, m.p. 224–231° (dec.).

Anal. Calcd. for $C_{10}H_{15}NO_5S$: C, 45.96; H, 5.79; N, 5.36. Found: C, 46.12; H, 5.64; N, 5.39.

Reductive Desulfurization of the Lactam-acid Vb.—The method employed was that used for the lower homolog. The filtered solution was extracted twice with ethyl acetate to give 35% of crude product that after several crystallizations from water and benzene–ethyl acetate was isolated as fine plates, m.p. 141.5–143.5°. A mixture of this and the material (m.p. 142.0–143.5°) obtained *via* 2-piperidone is 141.5–143.5°.

Anal. Calcd. for $C_{10}H_{17}NO_3$: C, 60.28; H, 8.60; N, 7.03. Found: C, 60.53; H, 8.65; N, 7.15.

α-1-(2-Piperidone)-isovaleric Acid.—This was prepared in a manner similar to that used to make the corresponding propionic acid except that the ethyl α-bromoisovalerate was added to the sodiopiperidone in boiling benzene, not to the cold suspension. The intermediate **ethyl ester**, b.p. 150–154° (0.3 mm.), was obtained in 48% yield; $n^{25}D$ 1.4735, d^{25}_4 1.0630; *MR* calcd. 60.45, *MR* obsd. 60.05.

Anal. Calcd. for $C_{12}H_{21}NO_3$: N, 6.16. Found: N, 6.49.

The ester was hydrolyzed by boiling with one equivalent of sodium hydroxide in methanol for one hour to give 43% of crude acid that after several crystallizations from benzene–heptane was obtained as colorless needles, m.p. 142–143.5°.

Anal. Calcd. for $C_{10}H_{17}NO_3$: C, 60.28; H, 8.60; N, 7.03. Found: C, 60.65; H, 8.60; N, 6.92.

Ethyl γ-bromocrotonate, prepared from ethyl crotonate by the use of *N*-bromosuccinimide and benzoyl peroxide in carbon tetrachloride,[24] was obtained in 84% yield, b.p. 117–120° (31 mm.), $n^{21}D$ 1.489.

γ-Bromocrotonic acid was prepared by barium hydroxide hydrolysis of the ester at low temperature, following Braun.[25] A product melting at 72–73° (reported m.p. 74°) was obtained in 45–50% yield. (This material is extremely irritating to the skin.)

The *p*-bromophenacyl ester, made in the usual way, was obtained, after several crystallizations from ethanol, as fine needles, m.p. 136.5–137.0° (some sintering at 133.5°).

Anal. Calcd. for $C_{12}H_{10}O_3Br_2$: C, 39.81; H, 2.78. Found: C, 40.09; H, 2.78.

γ-Phenylacetylaminocrotonic Acid.—A solution of γ-bromocrotonic acid (4.9 g., 0.030 mole) in about 100 cc. of liquid ammonia was placed in a Dewar flask and allowed to evaporate slowly. After the careful addition of 100 cc. of water the solution was evaporated dry on the steam-bath. Two equivalents of sodium hydroxide were added and the solution evaporated dry again. The crude material was taken up in a little water and treated with one equivalent of sodium hydroxide and one equivalent of phenylacetyl chloride, with cooling and stirring. The precipitate that formed was filtered and dried to give 5.5 g. (83%) of crude product, m.p. 153–154°. Further crystallization from water brought the melting point to 156.5–160.0°.

Anal. Calcd. for $C_{12}H_{13}O_3N$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.70; H, 5.95; N, 6.39.

When the γ-bromoester was treated in dry dioxane with liquid ammonia followed by phenylacetylation, no product could be isolated.

The *p*-bromophenacyl ester was prepared in the usual

(23) O. Wallach, *Ann.*, **321**, 171 (1900); "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, pp. 77, 371

(24) H. Schmidt and P. Karrer, *Helv. Chim. Acta*, **29**, 573 (1946).

(25) G. Braun, THIS JOURNAL, **52**, 3167 (1930).

manner. After three crystallizations from ethanol it was obtained as colorless crystals, m.p. 162.0–162.5°.

Anal. Calcd. for $C_{20}H_{18}O_4NBr$: C, 57.70; H, 4.36. Found: C, 57.74; H, 4.10.

Ethyl γ-Phenylacetylaminocrotonate.—A solution of 3.0 g. (0.014 mole) of the above acid in 120 cc. of absolute ethanol was treated with 5 cc. of acetyl chloride,[26] and left at room temperature for 38 hours. The solution was evaporated dry *in vacuo* with the minimum application of heat, and the residue dissolved in benzene. The benzene solution was washed with 10% sodium carbonate, water, saturated sodium chloride, and evaporated dry. There was left a solid that was washed with petroleum ether, filtered and dried to give 3.2 g. (92%) of the ester, m.p. 65–70°. Recrystallization from ether–petroleum ether gave 2.9 g. of colorless needles, m.p. 71.5–72.0°.

Anal. Calcd. for $C_{14}H_{17}O_3N$: C, 67.99; H, 6.95; N, 5.67. Found: C, 67.95; H, 6.82; N, 6.00.

This ester was prepared in poor yield and in less pure form when sulfuric acid was employed as the catalyst, or by the use of thionyl chloride followed by treatment with absolute ethanol.

Ethyl β-Chloro-γ-phenylacetylaminobutyrate.—A solution of γ-phenylacetylaminocrotonic acid in absolute ethanol was saturated with dry hydrogen chloride and gently refluxed for 2.5 hours. The ethanol was removed *in vacuo* and the residual gum dissolved in benzene, washed with 5% sodium carbonate, with water, and the benzene removed *in vacuo*. Treatment of the gummy residue with hexane gave a 70% yield of a crude solid, m.p. 45–50°. Recrystallization from ether–hexane gave a colorless product, m.p. 57.5–58.0°, which gave a negative permanganate test.

Anal. Calcd. for $C_{14}H_{18}O_3NCl$: C, 59.25; H, 6.39; Cl, 12.50. Found: C, 58.63; H, 6.23; Cl, 12.68.

Catalytic Reduction of γ-Phenylacetylaminocrotonic Acid.—A solution of 0.80 g. of pure γ-phenylacetylaminocrotonic acid in 50 cc. of ethanol was treated with 0.50 g. of platinum oxide and hydrogenated in an Adams–Parr type of apparatus at room temperature and 20 pounds pressure for two hours. The solution was filtered, taken dry *in vacuo*, and the residue dissolved in the minimum amount of boiling benzene (about 100 cc.). The cooled benzene solution deposited 0.67 g. of colorless microplates, m.p. 86–90°. A sample was recrystallized from ethyl acetate–heptane and from benzene to give fine plates, m.p. 93.0–94.0°.

Anal. Calcd. for $C_{12}H_{13}O_3N$: C, 65.14; H, 6.84; N, 6.33. Found: C, 65.33; H, 6.69; N, 6.35.

γ-Phenylacetylaminobutyric acid[27] was prepared in the usual manner from γ-aminobutyric acid, phenylacetyl chloride and 10% sodium hydroxide in good yield. The pure material crystallized from benzene to give microplates, m.p. 93.5–94.0°. A mixture of this material and the acid prepared *via* hydrogenation showed no melting point depression.

Anal. Calcd. for $C_{12}H_{15}O_3N$: C, 65.14; H, 6.84; N, 6.33. Found: C, 64.97; H, 6.61; N, 6.64.

α-Phenylacetylaminobutyric[28] acid was prepared for comparative purposes. Since we found it to melt at 127.0–129.5°, it was analyzed.

Anal. Calcd. for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.84; N, 6.33. Found: C, 64.94; H, 6.73; N, 6.42.

The N-benzoyl derivative[27] of γ-aminobutyric acid was similarly prepared, and recrystallized from benzene as fine needles, m.p. 79–80°.

Anal. Calcd. for $C_{11}H_{13}NO_3$: C, 63.65; H, 6.31. Found: C, 63.34; H, 6.19.

The N-benzenesulfonyl[27] derivative of γ-aminobutyric acid was similarly prepared and recrystallized from benzene; needles, m.p. 90.5–91.5°.

Anal. Calcd. for $C_{10}H_{13}NO_4S$: C, 49.82; H, 5.39. Found: C, 49.62; H, 5.53.

The N-*p*-toluenesulfonyl derivative[27] of γ-aminobutyric acid was similarly prepared and recrystallized from dilute ethanol; needles, m.p. 133.5–134.0°.

Anal. Calcd. for $C_{11}H_{15}NO_4S$: C, 51.34; H, 5.89; N, 5.45. Found: C, 51.18; H, 6.13; N, 5.59.

Attempted Preparation of Ethyl γ-Formylcrotonate.—Repetition of the procedure for formylating ethyl crotonate described by Borsche and Manteuffel[14] using ethyl formate and potassium in ether–ethanol solution gave, in good yield, a crude gum which gave a positive ferric chloride test and a positive Schiff test. However, the preparation of the 2,4-dinitrophenylhydrazone in the usual manner using an acid medium, resulted in very poor yields of an impure product of m.p. 137–142° (reported m.p. 147–148°).

When this crude oil was treated in ethanol with L-cysteine hydrochloride and one equivalent of sodium acetate, no thiazolidine could be isolated.

Various modifications in the above formylation were tried without success. Sodium in ether, in benzene, sodium ethoxide in benzene or ether[29] as well as variations in temperature and reaction time did not significantly improve the purity of the product.

Attempted Preparation of Ethyl γ-Formyl-γ-phenylacetylaminocrotonate (X).—Application to ethyl γ-phenylacetylaminocrotonate of the procedures used in the attempt to formylate ethyl crotonate gave no identifiable product, though a small yield of a crystalline 2,4-dinitrophenylhydrazone, m.p. 124–125°, was obtained. The analysis does not correspond to the expected product.

Anal. Found: C, 45.62; H, 4.66.

Attempted Condensation of Ethyl Formate with Ethyl γ-Bromocrotonate.—To a well-stirred solution of 9.8 g. (0.25 mole) of potassium dissolved in 180 cc. of dry ether and 45 cc. of absolute ethanol, was slowly added a mixture of 48 g. (0.25 mole) of ethyl γ-bromocrotonate and 20 g. (0.27 mole) of ethyl formate. After standing overnight the yellow precipitate present was filtered, dissolved in a little water and this solution washed twice with ether. The aqueous phase was ice-cooled, acidified, and extracted with ether to give 2.1 g. of a dark oil which failed to give a solid product on treatment with 2,4-dinitrophenylhydrazine solution. Repetition of the reaction using *t*-butyl alcohol in place of ethanol, and refluxing the reaction solution one-half hour likewise failed to give any detectable product.

Crotonaldehyde Diethylacetal.[30]—The method applied by Fischer and Baer[17] to the preparation of acrolein diethylacetal was employed. To a solution of 14.0 g. (0.20 mole) of crotonaldehyde in 36 g. of ethyl orthoformate was added 0.7 g. of ammonium nitrate in 25 cc. of ethanol, and the mixture refluxed ten minutes. The dark reaction mixture was cooled, filtered, diluted with ether, and washed with ammonia and with water. The dried ether solution was evaporated dry *in vacuo* and the residue distilled to give 23 g. (82%) of a faintly yellow liquid, b.p. 71° (43 mm.). This material had d^{25}_4 0.851, $n^{28}D$ 1.4039. The reported values are: d 0.846[30a]; $n^{18}D$ 1.4187.[30c]

Application of a modification due to Claisen[31] using ethyl orthoformate, absolute ethanol and ammonium chloride gave a 56% yield of a product with the same physical constants.

The 2,4-dinitrophenylhydrazone was prepared in the usual manner, m.p. 187–188°. It proved to be identical with the derivative prepared directly from crotonaldehyde.

Bromination of Crotonaldehyde Diethylacetal.—To a rapidly stirred solution of 40 g. (0.25 mole) of the acetal in 150 cc. of carbon tetrachloride was added 42.5 g. (0.25 mole) of N-bromosuccinimide portionwise at room temperature. No apparent reaction occurred even after the addition of a small amount of benzoyl peroxide. The suspension was refluxed gently, and after 15 minutes a vigorous reaction ensued. Heating was stopped until the reaction had subsided, and the solution then refluxed two hours, cooled, and filtered. The precipitate consisted of 16 g. (65%) of succinimide, m.p. 124–126°. After being cooled overnight the filtrate deposited no more succinimide. The solution was washed with 5% sodium bicarbonate, with water, and the solvent removed *in vacuo* on the steam-bath, with evidence of considerable decomposition of the product. The dark gummy residue was distilled at about 0.1 mm. to give

(26) K. Freudenberg and W. Jacobs, *Ber.*, **74B**, 1001 (1941).

(27) We are indebted to Mr. Peter Koromilas for the preparation of this compound.

(28) O. Behrens, *et al.*, *J. Biol. Chem.*, **175**, 759 (1948).

(29) Reference 5, pp. 506, 806, 807.

(30) (a) W. Hartung and H. Adkins, THIS JOURNAL, **49**, 2517 (1927); (b) W. Flaig, *C. A.*, **41**, 6189 (1947); (v) C. Harries and F. Düvel, *Ann.*, **410**, 69 (1915).

(31) L. Claisen, *Ber.*, **40**, 3903 (1907).

as the main fraction 11.1 g. (19%) of a liquid of b.p. 86–97°. There was a small forerun, and 8.0 g. of black tar remained behind. On redistillation in a molecular still there was obtained a fraction with $n^{25}D$ 1.4903 which gave a positive halogen test, a positive Baeyer test, and a negative Schiff test.[32]

The 2,4-dinitrophenylhydrazone was prepared in the usual manner. After recrystallization from ethanol it had m.p. 218–220° (dec.).

Anal. Calcd. for $C_{10}H_9N_4O_4Br$: N, 17.0; Br, 24.2. Found: N, 16.8; Br, 24.28.

N,N-Diphenylcrotonamide was prepared by a previously described method[33] involving the interaction of crotonyl chloride and diphenylamine in benzene. Recrystallization from dilute ethanol gave shiny colorless crystals, m.p. 114–115° (reported 113–114°,[33] 115–116°[34]). Bromination of the amide with N-bromosuccinimide in carbon tetrachloride in the presence of benzoyl peroxide gave none of the expected product.

N-Crotonylpiperidine was prepared by the same method to give 72% of an oil, b.p. 142.5° (21 mm.), $n^{26}D$ 1.5059, d^{26}_4 1.0006; *MR* obsd. 41.88, *MR* calcd. 44 92.

Anal. Calcd. for $C_9H_{15}ON$: C, 70.55; H, 9.87; N, 9.14. Found: C, 71.58; H, 9.78; N, 8.70.

The dibromide was prepared in good yield by the addition of bromine to a carbon tetrachloride solution of the compound. Recrystallization from dilute alcohol and then from hexane gave stout needles, melting constantly at 96.5–98.0°. The analysis shows the material still contains some impurity.

Anal. Calcd. for $C_9H_{15}ONBr_2$: C, 34.53; H, 4.83; Br, 51.06. Found: C, 34.90; H, 4.66; Br, 49.7.

Attempts to brominate N-crotonylpiperidine with N-bromosuccinimide in carbon tetrachloride with benzoyl peroxide catalyst led to the isolation of 70% of crude succinimide. Distillation *in vacuo* of the product, however, resulted in extensive decomposition and no identifiable product was isolated.

Ethyl γ-phthalimidocrotonate[35,36] was prepared in 70% yield by refluxing equimolar amounts of ethyl γ-bromocrotonate and potassium phthalimide in absolute ethanol for 24 hours. The product after several crystallizations from dilute ethanol and dilute acetone gave shiny plates, m.p. 96–97°.

Anal. Calcd. for $C_{14}H_{13}NO_4$: C, 65.11; H, 5.07; N, 5.40. Found: C, 65.17; H, 4.93; N, 5.4.

Hydrolysis of ethyl γ-phthalimidocrotonate with 6 *N* HCl for 24 hours gave a clear solution from which no solid could be isolated. Treatment of the crude product with phenylacetyl chloride and alkali led to the formation of a solid that after several crystallizations from ethanol is obtained as fine silky needles, m.p. 194.5–196.5°, identified as phenylacetylimide.[37]

Anal. Calcd. for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.81, 75.93; H, 5.71, 5.72; N, 5.45, 5.45.

(32) W. Flaig, *Ann.*, **568**, 24 (1950).

(33) N. Maxim, *Bull. soc. chim. Romania*, **10**, 97 (1928); *C. A.*, **23**, 2697 (1929).

(34) C. Bischoff, *Ber.*, **34**, 2135 (1901).

(35) The corresponding methyl ester has recently been prepared; W. Langenbeck and H. Boser, *Chem. Ber.*, **84**, 526 (1951).

(36) We are indebted to Mr. Alfred Haven for the preparation of this compound.

(37) O. Diels, *Ber.*, **36**, 747 (1903).

AMHERST, MASSACHUSETTS

[CONTRIBUTION FROM THE BOTANICAL INSTITUTE, FACULTY OF SCIENCE, UNIVERSITY OF TOKYO]

Anthochlor Pigments of *Cosmos sulphureus*, *Coreopsis lanceolata* and *C. saxicola*

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From the ray flowers of *Cosmos sulphureus*, a new anthochlor glycoside, sulphurein, has been isolated, and its constitution established as being the 6-glucoside of sulphuretin (3′,4′,6-trihydroxybenzalcoumaranone). Also isolated from the same plant was coreopsin, previously reported by Geissman and co-workers to be a glucoside of butein (3,4,2′,4′-tetrahydroxychalcone). The structure of coreopsin has now been determined to be the 4′-glucoside of butein. From the ray flowers of *Coreopsis lanceolata* and *C. saxicola*, a new chalcone glucoside, lanceolin, has been isolated. Lanceolin is considered to be the 4′-glucoside of lanceoletin (2′,4′,3,4-tetrahydroxy-3′-methoxychalcone). Leptosin, previously obtained by Geissman from *C. grandiflora*, has now also been obtained and identified from *C. lanceolata* and *C. saxicola*. Leptosin is the 6-glucoside of leptosidin (3′,4′,6-trihydroxy-7-methoxybenzalcoumaranone).

Introduction

Geissman and co-workers[1] isolated coreopsin, a butein glycoside, from the ray flowers of *Coreopsis gigantea* and *Cosmos sulphureus*, and leptosin, a glycoside of leptosidin, from the ray flowers of *Coreopsis lanceolata*.

We have also been, independently, engaged since 1948 in the study of yellow coloring matters in the flowers of some species of Coreopsis which are cultivated in Japan, such as *C. lanceolata L.*, *C. saxicola* Alexander and *Cosmos sulphureus Caviére*. We had isolated from the former two plants a chalcone glycoside and also an orange-yellow glycoside, and from the latter plant a butein glycoside and also an orange-yellow glycoside. Because of the shortage of foreign literature, which lasted from the outbreak of the War until several years after its end, we were unable to notice the works of Geissman until early in 1950. On reading the articles of Geissman, we became convinced that the butein glycoside from *Cosmos* and the orange-yellow glycoside from *Coreopsis*, isolated by us, are identical with coreopsin and leptosin, respectively.

Although the nature and position of the sugar residue in coreopsin had not been previously determined, Geissman thought that the sugar probably is attached to either the 2′- or 4′-position of butein. This suggestion was based on the observation that the red color of a solution of coreopsin in alkali is similar to that shown by other chalcones hydroxylated in the 3,4-positions, and that the color is deeper than those of solutions of 4-hydroxy- or 4-hydroxy-3-methoxychalcone in alkali.

Using the procedure detailed in the experimental section below, the sugar of coreopsin has now been proved to be glucose attached to the 4′-position of butein.

Paper chromatographic studies of the ethanol extract of the flowers of *Cosmos sulphureus* indicated

(1) T. A. Geissman, THIS JOURNAL, **63**, 2689 (1941); **64**, 1704 (1942); T. A. Geissman and C. D. Heaton, *ibid.*, **65**, 677 (1943); **66**, 486 (1944); T. A. Geissman and W. Mojé, *ibid.*, **73**, 5765 (1951).