the procedure of Weinstock and Boekelheide[36] did not lead to any recognizable products except for a small amount (*ca.* 5%) of recovered tetraphyllicine.

Selenium Dehydrogenation of Tetraphyllicine.—An intimate mixture of 300 mg. each of tetraphyllicine and black selenium was heated at 300° for 5 minutes, cooled, mixed with sand and extracted continuously for 18 hours in a Soxhlet extractor with benzene. The extract was stirred with mercury[37] for 4 hours, filtered through Celite powder and the residue after removal of the solvent was distilled at 130–140° and 0.015 mm. The yellow oil (75 mg.) which had distilled over was treated in ether solution with picric acid and the precipitate was recrystallized twice from methanol to yield 8 mg. of fine, yellow needles, m.p. 269–271° dec., undepressed upon admixture with an authentic specimen (m.p. 273–275° dec.) of *ind*-N-methylharman (IX) picrate[22,38]; the infrared spectra (potassium bromide pellet) were identical.

Acetylation of Tetraphyllicine.—A mixture of 100 mg. of tetraphyllicine, 4 cc. of benzene and 2 cc. of acetic anhydride was heated under reflux for 6 hours, concentrated *in vacuo* and diluted with ice-water. After making basic with ammonia, the product was extracted with ether and then with dilute hydrochloric acid, leaving a negligible residue in the ether solution. The acid extracts were again made basic, extracted with ether and the product was chromatographed on Merck acid-washed alumina. Apparently homogeneity was indicated by its chromatographic behavior (including infrared examination of most fractions eluted with chloroform and chloroform–methanol), but the substance could not be crystallized. It was then distilled at 160–165° and 0.002 mm. whereupon a heavy, colorless oil was obtained which crystallized rapidly; it melted partially at 60°, resolidified and yielded a clear melt at 154°; $\lambda_{max}^{CHCl_3}$ 5.78 and 8.0 μ. Since it could not be recrystallized satisfactorily, the distilled material was sent for analysis and while the latter was not very satisfactory, it indicated clearly (together with the relevant infrared bands) that the substance was O-acetyltetraphyllicine.

Anal. Calcd. for $C_{22}H_{26}N_2O_2$: C, 75.40; H, 7.48; N, 7.99; acetyl, 12.28. Found: C, 75.70; H, 8.19; N, 7.22; acetyl, 10.52.

Tetraphyllicine was obtained in excellent yield when the acetate was heated with an ethereal solution of lithium aluminum hydride or with 5% methanolic potassium hydroxide.

Ozonolysis of Tetraphyllicine.—Ozone was passed for 15 minutes through an ice-cold solution of 45 mg. of tetraphyllicine in 6 cc. of 2% acetic acid containing one drop of hydrochloric acid. The solution immediately turned deep purple and the excess ozone was swept out with nitrogen gas. The resulting dark brown solution was distilled in a current of nitrogen into an ice-cold aqueous sulfuric acid–ethanol solution of *p*-nitrophenylhydrazine yielding 15 mg. (55%) of acetaldehyde *p*-nitrophenylhydrazone with m.p. 124–125°, undepressed upon admixture with authentic material. No hydrazone formed in an appropriate blank experiment.

Hydrogenation of Tetraphyllicine (X) to Desoxyajmaline (VIII).[39]—A chloroform solution of 55 mg. of tetraphyllicine was treated with 5 cc. of hydrogen chloride–saturated chloroform and the solvent was removed. The resulting hydrochloride was hydrogenated at room temperature and atmospheric pressure in 95% ethanol solution with 25 mg. of prereduced platinum oxide catalyst whereupon one equivalent of hydrogen was consumed within one hour. Filtration of the catalyst, evaporation to dryness, addition of dilute ammonium hydroxide solution and isolation with chloroform yielded a crystalline residue which was recrystallized from methanol–acetone to furnish 43 mg. of fluffy, colorless needles showing m.p. 300–305°, undepressed upon admixture with an authentic specimen of desoxyajmaline (VIII).[20-22] The infrared spectra of the two specimens in Nujol mull were identical and the rotatory dispersion curves[40] (methanol solution) agreed within the acceptable 3% range inherent in rotation measurements at such dilute concentration.

Wave length, mμ	Tetraphyllicine (X) [α] (*c* 0.12)	Hydrogenated tetraphyllicine [α] (*c* 0.14)	Desoxyajmaline (VIII) [α] (*c* 0.075)
700	+94°	+74°	+75°
650	100	89	89
589	135	112	116
550	160	130	137
500	206	169	173
450	276	224	232
400	387	324	338
375	480	397	407
350	588	478	488
340	645	513	516
335	658	526	531
332.5	667	525	539
330	671	525	540
327.5	659	520	535

(36) J. Weinstock and V. Boekelheide, THIS JOURNAL, **75**, 2546 (1953).

(37) This method for removal of selenium was described recently by J. Jacques, G. Ourisson and C. Sandris, *Bull. soc. chim.*, 1293 (1955).

(38) R. B. Woodward and W. M. McLamore, THIS JOURNAL, **71**, 379 (1949).

(39) Poisson, Goutarel and Janot (ref. 28b) have described the hydrogenation of the rauvomitine (XI) saponification base to desoxyajmaline (VIII).

(40) For experimental details see C. Djerassi, E. W. Foltz and A. E. Lippman, THIS JOURNAL, **77**, 4354 (1955), and later papers in that series. We are indebted to Mrs. Rosemarie Riniker for the current measurements.

DETROIT, MICHIGAN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

The Synthesis of DL-Canaline, DL-Canavanine and Related Compounds[1]

BY DAVID D. NYBERG[2] AND BERT E. CHRISTENSEN

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A general discussion of the most satisfactory methods of preparation of O-substituted hydroxylamines and hydroxyguanidines is given. A new and practical synthesis of DL-canaline by a five-step reaction scheme from γ-butyrolactone in 7% over-all yield is described. DL-Canavanine is prepared directly from DL-canaline. An attempt to synthesize the lower homolog of DL-canaline resulted only in the isolation of DL-serine. An interesting material believed to be a polyoxime appeared as a product in this investigation.

In 1929, Kitagawa discovered the hitherto unknown amino acid canavanine ($NH_2C(=NH)NHOCH_2CH_2CH(NH_2)COOH$)[3] in the Jack bean meal from which he was extracting urease to be used in connection with his studies of the mechanism of urea formation in pig liver.

He reported that this new amino acid upon treatment with arginase[4] would yield urea and a second

(1) Published with the approval of the Monographs Publication Committee, Oregon State College as Research Paper No. 306, School of Science, Department of Chemistry.

(2) National Science Foundation Fellow 1954–1956.

(3) M. Kitagawa and T. Tomiyama, *J. Biochem.* (*Japan*), **11**, 265 (1929).

(4) (a) M. Damodaran and K. G. A. Narayanan, *Biochem. J.*, **34**, 1449 (1940); (b) M. Kitagawa, *J. Agr. Chem. Soc. Japan*, **15**, 267 (1939); *C. A.*, **34**, 1196 (1940).

amino acid canaline ($H_2NOCH_2CH_2CHNH_2COOH$).

The fact that canavanine was the first and, to the authors' knowledge, the only derivative of hydroxyguanidine to be found in nature has focused attention upon this compound. However, the main interest in canavanine stems from the fact that it has been shown to be a potent growth inhibitor of many organisms.[5] This growth inhibition may be due to the competitive antagonism of arginine, which is not surprising in view of the close structural relationships between the two amino acids.

Canavanine, for example, has been found to inhibit the multiplication of the Lee influenza virus in the chick embryo and in tissue culture.[6] It has also been shown to be inhibitory to the growth of the mouse encephalomyelitis virus in tissue culture.[7]

It was such biological interest in canavanine that provided the stimulus for the work reported in this paper, and some of the synthetic compounds described herein were prepared for biological testing.

The only reported work on the synthesis of canavanine is that of Kitagawa who prepared this amino acid[8] by a tedious method from the canaline obtained by the enzymatic cleavage of L-canavanine itself.[4] For obvious reasons this was not a practical procedure for the preparation of L-canavanine but was undertaken in a effort to establish the structure of this interesting amino acid. No yields were reported by Kitagawa, and the directions given are lacking in clarity and detail.

Kitagawa also established the relationship of L-canaline and L-α-amino-γ-hydroxybutyric acid[9] by showing the one to be the hydrogenation product of the other. Then starting with α-amino-γ-hydroxybutyric acid he succeeded in a five-step sequence of reactions (in which no yields were reported) in preparing canaline.

Borek and Clarke[10] attempted several methods of synthesis but were unable to prepare canaline by any of the procedures used. Their starting material was acetone β-bromoethoxime, prepared from the sodium salt of acetone oxime and ethylene bromide. These workers were able to prepare several aminoöxy compounds; however, the closest to the structure of canaline was γ-carboxypropoxyamine (4-aminoöxybutanoic acid).

In this study the starting material for the synthesis of canaline was γ-butyrolactone (see Fig. 1). It possesses a preformed four-carbon chain system which hence makes unnecessary any complicated carbon-to-carbon condensations and lends itself readily to the reactions involved. This lactone was brominated according to the directions of Livak, *et al.*,[11] to give an 89% yield of α-bromo-γ-butyrolactone, which was treated with 28% aqueous ammonia in a similar manner to that of Plieninger[12] to yield (68%) α-amino-γ-butyrolactone hydrobromide. Opening the lactone ring according to Fischer and Blumenthal[13] gave an 84% yield of *dl*-2-amino-4-hydroxybutanoic acid (DL-homoserine).

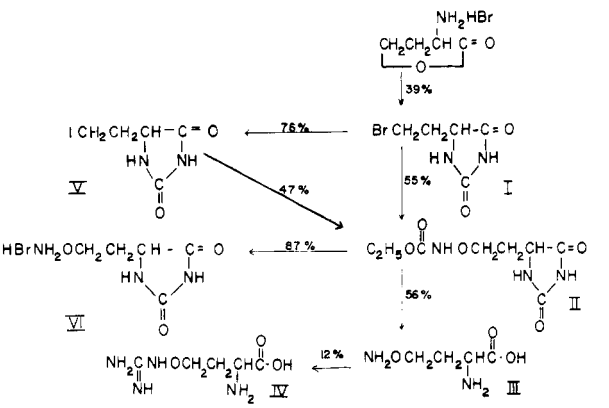

Fig. 1.

To introduce an aminoöxy group on the C_4-atom of the lactone (or homoserine) required a prior introduction of a bromine or iodine atom, while at the same time protection of the α-amino group and inactivation of the carboxyl group were desired; 5-(2-Bromoethyl)-hydantoin (I) meets such requirements. Livak[11] had prepared this compound from homoserine, in 51% yield. The average yield obtained in this Laboratory was only 42%. However, it was possible to prepare it directly from the lactone in 39% yield by merely neutralizing the hydrogen bromide with the calculated amount of potassium carbonate and proceeding directly to the synthesis of 5-(2-bromoethyl)-hydantoin without the isolation of homoserine. This resulted in a small increase in the total yield of the hydantoin from γ-butyrolactone. The bromo compound was easily converted to 5-(2-iodoethyl)-hydantoin (V) in 76% yield by sodium iodide in acetone.

As a means of introducing the aminoöxy group, hydroxyurethan[14] was used in alcoholic potassium hydroxide to give a 55% yield of 5-[2-(carbethoxyaminoöxy)-ethyl]-hydantion (II).[15,16] In contrast to some of the other methods, no ambiguous products or isomers have been reported in the literature by workers using this procedure. Furthermore the homogeneous reaction medium simplifies observing the progress of the reaction *via* the precipitation of the potassium halide side product. This reaction was studied quite thoroughly, and it was found that a ratio of hydroxyurethan to 5-(2-bromoethyl)-hydantoin of 2:1 gave the best results. A 1:1 ratio resulted in only an 11% yield and a 3:1 ratio gave no increase in yield (51%); almost identical results were obtained using 5-

(5) (a) B. Volcani and E. Snell, *J. Biol. Chem.*, **174**, 893 (1948); (b) N. H. Horowitz and A. Srb, *ibid.*, **174**, 371 (1948); (c) T. Suzuki, S. Muraoka and K. Konobu, *J. Pharm. Soc. Japan*, **74**, 534 (1954); *C. A.*, **48**, 16116 (1954); J. B. Walker, *J. Biol. Chem.*, **212**, 207 (1955).

(6) K. S. Pilcher, *et al.*, *Proc. Soc. Exp. Biol. Med.*, **88**, 79 (1955).

(7) H. E. Pearson, D. L. Lagerborg and R. J. Winzler, *ibid.*, **79**, 409 (1952).

(8) M. Kitagawa and A. Takami, *J. Biochem. (Japan)*, **23**, 181 (1936).

(9) M. Kitagawa, *ibid.*, **24**, 107 (1936).

(10) E. Borek and H. T. Clarke, *J. Biochem.*, **125**, 479 (1938).

(11) J. E. Livak, *et al.*, THIS JOURNAL, **67**, 2218 (1945).

(12) H. Plieninger, *Ber.*, **83**, 265 (1950).

(13) E. Fisher and H. Blumenthal, *ibid.*, **40**, 106 (1907).

(14) A. T. Fuller and H. King, *J. Chem. Soc.*, 963 (1947).

(15) D. Nyberg and B. E. Christensen, THIS JOURNAL, **78**, 781 (1956).

(16) (a) L. W. Jones, *Am. Chem. J.*, **20**, 1 (1898); (b) C. H. Hecker *ibid.*, **50**, 444 (1913).

(2-iodoethyl)-hydantoin. The use of potassium ethoxide in anhydrous ethanol was tried using a 1:1 ratio; very crude material in a 26% yield was obtained.

DL-Canaline (III) was obtained in 56% yield from 5-[2-(carbethoxyaminoöxy)-ethyl]-hydantoin by hydrolysis with 13.5% barium hydroxide for 12 hr. at reflux temperature. The canaline thus obtained was isolated as its picrate from which it was recovered in quantitative amounts.

DL-Canaline was converted to DL-canavanine (IV) by the use of methylisourea hydrochloride according to the method of Kurtz[17]; however, a yield of only 10–12% was obtained. The reason for these low yields is not known to the authors. Slight variations in reaction conditions such as ratio of concentrations of reactants and length of reaction time did not affect the yields greatly. Methylisothiourea sulfate was also used successfully but in only one run. This procedure is not applicable to the synthesis of an optically active form of canaline because of rapid racemization of the hydantoin intermediate in alkaline solution.[18] The over-all yield of canaline from γ-butyrolactone was 7% and that of canavanine 0.9%.

It was found that 5-[2-(carbethoxy-aminoöxy)-ethyl]-hydantoin could be hydrolyzed with 48% hydrobrobromic acid to 5-[2-(aminoöxy)-ethyl]-hydantoin hydrobromide (VI). This material was treated with methylisothiourea sulfate in an endeavor to introduce the guanidinoöxy group. The product from this operation, without characterization, was immediately treated with 13.5% barium hydroxide for 12 hr. and the hydrolysate worked up in a manner similar to that for canavanine. Only canaline monoflavianate was isolated, however. It was not possible to tell from this result whether the aminoöxy hydantoin had been guanylated or not. Such a compound may have been degraded, under the alkaline conditions used in the hydrolysis to regenerate the aminoöxy compound. It had been hoped that such a guanidinoöxy compound would be stable, under such alkaline conditions, in view of the known alkaline stability of canavanine.[19]

One of the early objectives of this study was the synthesis of the lower homolog of canaline. For this purpose it was decided to employ the Strecker reaction to make the α-amino acid and the oxime method of Borek and Clarke[19] to introduce the aminoöxy group.

The acetal, acetone O-[2,2-(diethoxy)-ethyl)-oxime (VII) (see Fig. 2) was prepared in 51% yield from the sodium salt of acetone oxime and bromoacetal[20] in dry toluene. (An attempt to substitute commercially available chloroacetal for bromoacetal was unsuccessful.) This acetal was hydrolyzed with 1% hydrochloric acid in the presence of a large excess of acetone. This liberated the aldehyde group but retained the oxime linkage to give very crude acetone O-formylmethyloxime (VIII).

From the analytical data, it appears that acid hydrolysis in the absence of acetone caused a hydrolytic cleavage of the oxime to initially form 2-aminoöxyacetaldehyde, NH_2OCH_2CHO, which polymerized in a head-to-tail fashion to give a polyoxime (X).

Having obtained the crude aldehyde (VIII) in good yield, a Strecker reaction was run in an attempt to produce the desired amino acid. As is well known, a strong mineral acid is used to hydrolyze the α-amino nitrile intermediate in this synthesis; since only DL-serine (IX) was isolated from the reaction, it was concluded that the acid hydrolysis resulted in the degradation of the aminoöxy group to the hydroxyl group. Such a result was not completely surprising since Sidgwick[21] mentioned that O-ethylhydroxylamine was hydrolyzed by hydrochloric acid at 150° to ethyl chloride and hydroxylamine.

An attempt to utilize a hydantoin intermediate according to Holland and Nayler[22] to make the desired amino acid was also unsuccessful.

Experimental

5-(2-Bromoethyl)-hydantoin (I).—α-Amino-γ-butyrolactone hydrobromide[12] (27.3 g., 0.15 mole) was dissolved in 90 ml. of water, and anhydrous potassium carbonate (10.4 g., 0.075 mole) added with stirring. The slightly alkaline solution was heated on the steam-bath for a few minutes, and then a solution of potassium cyanate (13.0 g., 0.16 mole) in 50 ml. of water was added. The final solution was heated on the steam-bath for 2 hr. The rest of the procedure was carried out according to Livak, *et al.*, who used DL-homoserine for this preparation. The solution was treated with 100 ml. of 48% hydrobromic acid and heated for an additional 2 hr. on the steam-bath. The solution was evaporated to dryness *in vacuo* and the residue digested with 150 ml. of hot acetone and filtered. The potassium bromide residue was washed until white with hot acetone. The acetone filtrate was evaporated and the residue heated for 2 hr. on the steam-bath with another 100 ml. of 48% hydrobromic acid. After evaporating *in vacuo*, the residue was dissolved in 75 ml. of hot water, brought to *p*H 5–6 with concentrated ammonia, filtered and cooled. The crude crystalline product was removed 48 hr. later and immediately recrystallized from 70 ml. of water; yield 6.5–12.1 g. (21–39%) which melted at 139.5–40.0°. A mixed melting point with the same product prepared from DL-homoserine showed no depression.

5-(2-Iodoethyl)-hydantoin (V).—5-(2-Bromoethyl)-hydantoin (I) (2.07 g., 0.010 mole) and sodium iodide (1.80 g., 0.012 mole) were dissolved in 30 ml. of acetone and the solution refluxed for 0.5 hr. An additional 20 ml. of acetone was added, the solution cooled to room temperature and potassium bromide removed by filtration. The filtrate was evaporated to remove acetone and the residue taken up in 35 ml. of hot water, filtered and cooled to precipitate the product as tan colored tiny crystals; yield 1.93 g. (76%), m.p. 168.0–169.5°. Two recrystallizations from water gave tiny white scales melting at 172.5–173.0°.

Anal. Calcd. for $C_5H_7IN_2O_2$: C, 23.6; H, 2.8; N, 11.0. Found: C, 23.2; H, 2.7; N, 11.0.

5-(2-(Carbethoxyaminoöxy)-ethyl)-hydantoin (II).—To an alcoholic solution of potassium hydroxide made from 3.96 g. (0.06 mole) of 85% potassium hydroxide and 60 ml. of absolute alcohol was added 6.21 g. (0.03 mole) of 5-(2-bromoethyl)-hydantoin (I) and a solution of 6.30 g. (0.06 mole) of hydroxyurethan[14] in 40 ml. of absolute alcohol. On warming, a light brown solution was obtained which was refluxed for 3 hr., cooled and filtered to remove potassium bromide. The filtrate was evaporated to dryness and the sirupy residue dissolved in 30 ml. of water, neutralized with dilute hydrochloric acid and placed in the refrigerator; 24

(17) A. C. Kurtz, *J. Biol. Chem.*, **180**, 1253 (1949).

(18) H. D. Dakin, *Biochem. J.*, **13**, 398 (1919).

(19) M. Kitagawa, *J. Biochem. (Japan)*, **25**, 23 (1937).

(20) S. M. McElvain and D. Kundiger, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., pp. 123–125.

(21) N. V. Sidgwick, T. W. J. Taylor and W. Baker, "The Organic Chemistry of Nitrogen," Rev. Ed., Oxford, Clarendon Press, 1937, p. 161.

(22) D. O. Holland and J. H. C. Nayler, *J. Chem. Soc.*, 3403 (1952).

to 48 hours later a crop of white needles (2.88 g., m.p. 151.5–153°) was removed. (Sometimes no crystals appear.) The mother liquor was evaporated on the steam-bath to one-half of its original volume and set in a refrigerator overnight. The product was removed and the mother liquors exhaustively extracted with ether. The ether extract was evaporated to one-fourth of its original volume and cooled to give additional product (m.p. 150–153°), over-all yield 2.0–3.6 g. (52%). Recrystallization from water gave colorless needles melting at 152–153°.

Anal. Calcd. for $C_8H_{13}N_3O_5$: C, 41.6; H, 5.7; N, 18.2. Found: C, 41.3; H, 5.8; N, 18.4.

Experiments with 5-(2-iodoethyl)-hydantoin did not improve the yield nor did decreasing the ratio of 5-(2-bromoethyl)-hydantoin to hydroxyurethan from 1:2 to 1:3. A 1:1 ratio resulted in only an 11% yield of a very crude product.

5-(2-(Aminoöxy)-ethyl)-hydantoin hydrobromide (VI).—5-(2-(Carbethoxyaminoöxy)-ethyl)-hydantoin (2.31 g., 0.01 mole) was refluxed in 4.2 ml. of 48% HBr for 2 hr. The brown hydrolysate was immediately evaporated to dryness *in vacuo* and the residue thoroughly dried by adding a little absolute alcohol and evaporating once again. The residue from this operation was triturated with a small amount of absolute alcohol and finally placed in the refrigerator. Brown colored crystals were then recovered and washed with ether; yield 2.09 g. (87%). A recrystallization from 95% ethyl alcohol gave tan colored crystals, m.p. 167–168° dec.

Anal. Calcd. for $C_5H_{10}O_3N_3Br$: C, 25.0; H, 4.2; N, 17.5. Found: C, 24.7; H, 4.3; N, 17.5.

DL-Canaline (DL-2-Amino-4-aminoöxybutanoic Acid) (III).—5-(2-(Carbethoxyaminoöxy)-ethyl)-hydantoin (2.31 g., 0.01 mole) and barium hydroxide octahydrate (18.15 g.) were refluxed in 55 ml. of water for 12 hr. The white solid residue was removed and the filter cake extracted with 25 ml. of boiling water and finally washed with 25 ml. of hot water. The combined filtrate and washings were treated with 5.7 g. of ammonium carbonate by heating and stirring, the barium carbonate was removed and washed with hot water and the filtrate and washings were evaporated to dryness *in vacuo*. Attempts to bring about crystallization of the material obtained at this stage were unsuccessful. Picric acid (5.4 g., 0.02 mole) was added with 100 ml. of water, and the mixture was heated to effect solution, filtered and allowed to crystallize at room temperature. Tiny yellow needles of **DL-canaline dipicrate** were obtained, removed and washed with a little water; yield 3.38 g. (57%) melting at 189.5–191.5° with slight sintering at 187.5°. Recrystallization from water gave crystals melting at 190.5–191.0°.

Anal. Calcd. for $C_{16}H_{16}N_8O_{17}$: C, 32.45; H, 2.7. Found: C, 32.45; H, 2.9

In order to prepare free canaline, the dipicrate (14.6 g., 0.0247 mole) was decomposed with 100 ml. of hot 10% sulfuric acid. After cooling in a refrigerator overnight, the picric acid was removed and washed with a little ice-water. In order to remove all traces of picric acid from the filtrate and washings, they were combined and extracted exhaustively with ether. The now colorless solution was diluted to 450 ml. and treated with barium hydroxide octahydrate to quantitatively remove sulfate ion. The barium sulfate was removed and washed with a little hot water. The filtrate and washings were evaporated to near dryness *in vacuo* and 20 ml. of isopropyl alcohol added to the residue followed by refrigeration; yield 3.18 g. (96% from picrate) of white crystals melting at 198–201° dec. Two recrystallizations from aqueous isopropyl alcohol gave white needles, m.p. 195–198° dec.

A paper chromatogram using the butanol–acetic acid–water (4:1:5) system gave an R_f value of 0.59 and indicated only slight traces of impurity. The synthetic product gave a positive ninhydrin test and an orange-red color with alkaline picrate (Jaffe's test) which is characteristic of canaline.

Anal. Calcd. for $C_4H_{10}N_2O_3$: C, 35.8; H, 7.5; N, 20.9. Found: C, 35.9; H, 7.8; N, 20.7.

DL-Canaline monoflavianate, prepared from equimolar quantities of canaline and flavianic acid in water, was obtained as yellow crystals which melted at 201–202.5°. Recrystallization from water raised the melting point to 207–208°.

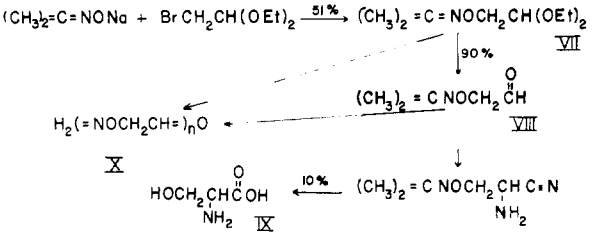

Fig. 2

Anal. Calcd. for $C_{14}H_{18}N_4O_{11}S$: C, 37.5; H, 3.6. Found: C, 37.6; H, 3.5.

DL-Canavanine (DL-2-Amino-4-guanidinoöxybutanoic Acid) (IV).—Canaline (III) (0.67 g., 0.005 mole) was converted to its copper complex with 0.72 g. of cupric carbonate or 0.44 g. of cupric oxide by boiling the reactants in water for 10 minutes and then filtering. The deep blue solution was evaporated to 7–8 ml. on the steam-bath. After cooling in an ice-bath, methylisourea hydrochloride[17] (1.1 g., 0.01 mole) and 5.0 ml. of 2 *N* sodium hydroxide were added. The solution was allowed to sit at room temperature for two weeks. At the end of this time, it was noted that crystallization of a purple-pink material had occurred and the *p*H of the solution had decreased from approximately 10 to 8. The solution was acidified to congo red with dilute hydrochloric acid (purple-pink crystals dissolved), treated with hydrogen sulfide and filtered. The filtrate was brought to boiling to remove hydrogen sulfide. (At this point Kitagawa[19] refluxed his synthetic product for 20 hr. with 10% hydrochloric acid to remove a contaminant. Similar treatment of this product gave no improvement in yield or quality.)

Flavianic acid dihydrate (1.75 g.) dissolved in a little warm water was added and the solution placed in the refrigerator. The next day yellow crystals of a crude canavanine flavianate were collected (1.90 g.). This was immediately recrystallized from water to remove a high-melting contaminant of unknown constitution; yield 1.22 g., m.p. 200–220° dec. Satisfactory analytical data for this compound were not obtained, but the results suggest the material to be a monoflavianate.

This flavianate without further characterization was dissolved in 40 m. of hot water and treated with 1.13 g. of barium hydroxide octahydrate dissolved in boiling water. On cooling, the precipitated barium flavianate was removed and washed twice with hot water. The still yellow filtrate was treated with sulfuric acid to quantitatively remove barium and finally charcoaled to remove the last traces of flavianic acid. The now colorless solution was evaporated to dryness *in vacuo*, and to the residue was added 20 ml. of absolute alcohol. White to slightly gray crystals separated which melted at 180–184° dec. on rapid heating. An authentic sample of L-canavanine prepared from Jack bean meal gave a melting point of 180–182° dec. on fast heating, and a mixed melting point showed no depression of temperature; yield 0.09–0.11 g. (10–12%).

The synthetic material gives a positive ninhydrin test and a positive canavanine test with amidine–pentacyanoferrate reagent.[23]

Comparative paper chromatograms from two different solvent systems of the synthetic and natural products as well as mixed material gave identical R_f values within each system. The R_f values obtained from the butanol–acetic acid–water (4:1:5) system were 0.054 and 0.077 in two different runs while the phenol–water (80% phenol) system gave an R_f of 0.47.

Acetone O-(2,2-(Diethoxy)-ethyl)-oxime (VII).—The sodium salt of acetone oxime (0.4 mole) was prepared in the same manner as was done previously in the preparation of acetone O-ethyloxime. The dried material was made into a slurry with 160 ml. of dry toluene and placed aside in a beaker temporarily. To the same flask (1000 ml., 3-necked) used in the preparation of the oxime salt were fitted a stirrer and reflux condenser with a drying tube. Bromoacetal[20] (160 g., 0.81 mole) and toluene (40 ml.) were introduced and

(23) W. R. Fearon, *Analyst*, **71**, 562 (1946).

heated by means of an oil-bath at 135°. The slurry of sodium acetone oxime was added in small portions over 1 hr. and the subsequent mixture stirred and heated for 24 hr. After cooling, the reaction mixture was filtered to remove the insoluble solids. The brown filtrate was distilled through a 42-cm. Vigreux column and the fraction boiling above 92° (20 mm.) collected. The lower boiling portion was distilled again and additional material boiling above 92° (20 mm.) collected. Approximately 40–50 g. was accumulated in this manner. Two more distillations gave 22–38 g. (29–51%), b.p. 40–45° (1.5 mm.), 98–99° (20 mm.), $n^{25}D$ 1.4259, of a colorless oil.

Anal. Calcd. for $C_9H_{19}NO_3$: C, 57.1; H, 10.1. Found: C, 56.5; H, 9.9.

The unchanged bromoacetal can be recovered from the lower boiling distillates with little trouble

A. The acid hydrolysis of acetone O-(2,2-(diethoxy)-ethyl)-oxime (VII) in the presence of an excess of acetone: In a solution of 35 ml. of acetone and 19 ml. of 1% hydrochloric acid were dissolved 2 g. (0.0212 mole) of acetone O-(2,2-(diethoxy)-ethyl)-oxime (VII). After refluxing for 1 hr., the solution was cooled in an ice-bath and then neutralized with anhydrous potassium carbonate to *p*H 7.5–8.5. The acetone was removed by evaporation *in vacuo* at temperatures not over 45°. The residue was chilled in an ice-bath and extracted with three to four portions of ether. Anhydrous sodium sulfate was used as a salting-out agent to ensure complete extraction. The ether extracts were dried with anhydrous sodium sulfate. After evaporation of the ether, 1.3–2.5 g. of a slightly yellow oil was obtained. This crude product decomposed even on vacuum distillation, and it was not possible to obtain pure material. However, the oil gave the typical tests expected for an aldehyde (Schiff and Tollens tests) and is probably crude **acetone O-formylmethyloxime (VIII)**. Attempts to prepare several aldehyde derivatives were all unsuccessful. Treatment with dilute aqueous hydrochloric acid gave a white insoluble precipitate similar in its thermal properties to the polymeric material described in part B.

B. The acid hydrolysis of acetone O-(2,2-diethoxy-ethyl)-oxime with aqueous hydrochloric acid in the absence of acetone: When the acetal, acetone O-(2,2-(diethoxy)-ethyl)-oxime (VII), was hydrolyzed in 1:1 or 2:1 1% aqueous hydrochloric acid–95% ethanol in the absence of acetone by refluxing for 15–60 minutes, a cream colored to white gummy insoluble material was deposited. On drying over P_2O_5 *in vacuo*, it became more friable and amorphous. Two grams of the acetal gave about 0.55 g. of this material. The sample analyzed below melted over the range 90–190° dec. and gave positive Schiff and Tollens tests.

Anal. Calcd. for $C_2H_3NO(=NOCH_2CH=)$: C, 42.4; H, 5.3. Found: C, 42.9; H, 5.9.

This analysis suggests the material to be polymeric in nature and composed of the units $=NOCH_2CH=$ since the carbon and hydrogen content of possible monomeric products are much higher than that found for the material isolated.

Serine (IX).—The attempted synthesis of 3-aminoöxy-2-aminopropanoic acid *via* the Strecker reaction and the isolation of serine (IX): Material believed to be crude acetone O-formylmethyl-oxime (VIII) (4.1 g., 0.0356 mole) prepared as described above was added to 7.2 g. of 10% methanolic ammonia. To the resulting solution was added ammonium chloride (2.86 g., 0.534 mole) dissolved in 17 ml. of water. The mixture was heated at 80° for 4 hr. The dark red solution was cooled and then added to 50 ml. of concentrated hydrochloric acid (hood) and allowed to sit overnight. The next day, the solution was heated on the steam-bath for 3.5 hr.; additional acid (20 ml.) was added and heating continued for 6 hr. longer. The solution was then evaporated to dryness *in vacuo* and the residue redissolved in water and filtered to remove a black insoluble material. The clear brown colored filtrate was passed through an IRA-400 strongly basic anionic exchange resin to remove the anions from the solution. The resin was then eluted with 2 *N* hydrochloric acid to give an amino acid effluent which was identified by means of the ninhydrin test. The fractions containing the amino acid were evaporated to dryness and treated with silver carbonate (0.1068 equiv.) to remove chloride. After removing silver chloride, the filtrate was saturated with hydrogen sulfide and filtered again. The final solution was evaporated to dryness and 50 ml. of absolute alcohol added. The cloudy solution was placed in the refrigerator. The next day a slightly yellow crystalline material was removed and immediately recrystallized from water–isopropyl alcohol. A crystalline material, 0.46 g. (10.3% yield of serine), m.p. 175–275° dec., was thus obtained. The material was again recrystallized from water–isopropyl alcohol.

A paper chromatogram using the phenol–water system with an authentic sample of serine showed the material to be predominantly serine with only a trace of some other material which also gave a ninhydrin test.

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Substitution of Polynuclear Aromatic Compounds. I. The Friedel–Crafts Benzoylation of Naphthalene

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Excess benzoyl chloride added to the reaction mixture does not change the rate of the aluminum chloride-catalyzed benzoylation of toluene or *p*-xylene or the amount of *ortho* isomer (9.7%) formed in the reaction with toluene. In the reaction with naphthalene, the addition of excess benzoyl chloride to the reaction mixture causes a large decrease in the rate of reaction and an increase in the amount of β-ketone (less hindered) formed. It has been suggested previously that the change in isomer distribution in the reaction with naphthalene is due to the excess benzoyl chloride complexing with the attacking group to form a more bulky group. The results indicate that this postulate can be true only if the attacking groups are different in the reactions with toluene (and *p*-xylene) and naphthalene. It is shown that rearrangement is not responsible for the differences in isomer distribution.

Introduction

In 1886, Claus and Feist[1] reported that the α-ketone is exclusively formed in the Friedel–Crafts acetylation of naphthalene. Ten years later Rousset[2] reported that both the α- and β-isomers are formed. Since that time the isomer distribution in this reaction has been investigated by numerous workers, but many of the results are confusing and contradicting.[3] It is now recognized that the addition of a variety of substances, which complex strongly with aluminum chloride, to the reaction

(1) A. Claus and P. Feist, *Ber.*, **19**, 2896 (1886).

(2) L. Rousset, *Bull. soc. chim.*, **15**, 58 (1896).

(3) An excellent review of the experimental work is given in the A.C.S. Monograph, C. A. Thomas, "Anhydrous Aluminum Chloride in Organic Chemistry," Reinhold Publ. Corp., New York, N. Y., 1941, p. 271. Reviews are also given by G. Lock, *Monatsh.*, **74**, 77 (1943), and by H. F. Bassilios, S. M. Makar and A. Y. Salem, *Bull. soc. chim.*, 72 (1954).