at 75–80° for six hours. It became dark and the sugar dissolved after three hours. The mixture was evaporated at 25° and atmospheric pressure yielding 24.5 g. of dark brown, partially crystalline paste. Recrystallization from Skellysolve B gave 0.25 g. of faintly tan solid, m.p. 210–220° dec., insoluble in water. Analysis indicated it was a mixture of N-dodecylmaltosylamine (or an isomer) with a larger amount of the reaction product derived from two moles of amine.

*Anal.* Calcd. for $C_{24}H_{47}NO_{10}$: C, 56.58; H, 9.30; N, 2.75. Calcd. for $C_{36}H_{73}N_2O_9$: C, 63.77; H, 10.85; N, 4.13. Found: C, 61.33; H, 10.19; N, 4.42.

**N-Octadecyllactosylamine.**—Lactose monohydrate (19.8 g., 0.055 mole) and octadecylamine (26.9 g., 0.10 mole) were dissolved in a mixture of isopropyl alcohol (200 ml.) and water (120 ml.). After a day at 25°, it had largely solidified. It was warmed to 60°, becoming a clear solution, allowed to cool and crystallize, and filtered, giving 36.9 g. of white solid, m.p. 106.5–108.5°. It was recrystallized twice from absolute ethanol, m.p. 119–121.5° dec.

*Anal.* Calcd. for $C_{30}H_{59}NO_{10}$: C, 60.67; H, 10.02; N, 2.36. Found: C, 59.60; H, 10.46; N, 2.67.

**Reaction of Lactose with Two Moles of Octadecylamine.**—A mixture of octadecylamine (26.9 g., 0.10 mole), lactose (7.2 g., 0.02 mole), water (25 ml.) and isopropyl alcohol (75 ml.) was heated at 70° for one hour. The sugar soon dissolved and the mixture became colored. After cooling, it could not be filtered so the entire mass was air-dried, 31.9 g., m.p. 70–85°. Three recrystallizations from alcohol gave a yellow solid, m.p. 116–117°. Analysis indicated it was a mixture of N-octadecyllactosylamine (or isomer) and a product derived from two moles of amine.

*Anal.* Calcd. for $C_{30}H_{59}NO_{10}$: C, 60.67; H, 10.02; N, 2.36. Calcd. for $C_{48}H_{96}N_2O_9$: C, 68.19; H, 11.44; N, 3.31. Found: C, 63.80; H, 10.34; N, 3.07.

MINNEAPOLIS 13, MINNESOTA

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

# Isolation, Structure and Synthesis of a Lathyrus Factor from *L. Odoratus*[1,2]

BY E. D. SCHILLING AND F. M. STRONG

RECEIVED JANUARY 3, 1955

The crystalline substance $C_8H_{13}O_3N_3$, isolated from *Lathyrus odoratus* seeds, which produces in rats the skeletal deformities characteristic of lathyrism, has been shown by degradation and synthesis to be β-(N-γ-L-glutamyl)-aminopropionitrile.

The isolation from *Lathyrus odoratus* seeds of a crystalline substance capable of producing skeletal abnormalities characteristic of lathyrism in rats has recently been accomplished.[3–6] The substance I, obtained in this Laboratory, fine white needles, m.p. 193–194° dec., gave analytical values agreeing with the formula $C_8H_{13}O_3N_3$.[4]

The compound gave one well-defined ninhydrin-positive spot, with no evidence of admixture with any ninhydrin-positive impurity, when subjected to paper chromatography in three different solvent systems according to published procedures. After strong acid hydrolysis this spot disappeared and was replaced by two others. This behavior plus the amphoteric and strongly polar character of the substance suggested that I could well be a dipeptide. Concentration of the hydrolysis mixture yielded a crystalline degradation product which was identified as L-glutamic acid hydrochloride. When the filtrate was made alkaline, the odor of a volatile base became evident. The volatile material was aerated into dilute hydrochloric acid and the resulting hydrochloride found to be free of carbon. The volatile base, therefore, was ammonia. Since the non-volatile portion of the hydrolysate still showed two ninhydrin spots in paper chromatograms, at least one other nitrogenous degradation product remained to be identified.

Calculations based on the $C_8H_{13}O_3N_3$ formula revealed unsaturation in I equivalent to four double bonds. Since the glutamic acid accounted for two of these, it was obvious that some other structure, probably in the unidentified three carbon portion of I, contained the remaining unsaturation. Furthermore, it appeared that in the original molecule this three-carbon portion must have contained either one or two nitrogen atoms.

Consideration was therefore directed to type structures for the remaining fragment which would contain three carbon atoms, one or two nitrogen atoms, two double bonds or the equivalent, and a grouping which would yield ammonia on hydrolysis. In this connection it was observed that the infrared spectrum of I showed a sharp band at 4.45 μ characteristic of a triple bond. This infrared band, the production of ammonia on hydrolysis, and the expected degree of unsaturation were all compatible with the presence of a nitrile function in I. On the assumption that a nitrile group was in fact present, the unidentified hydrolysis product would have had to be either alanine, β-alanine or sarcosine. Comparison with known samples on paper chromatograms clearly pointed to β-alanine as the actual degradation product, and its presence in the hydrolysate of I was then verified by direct isolation of a crystalline derivative.

In the light of the above results it seemed probable that I was a peptide-like combination of either glutamic acid plus β-aminopropionitrile or β-alanine plus a glutamonitrile. Of the six possible structures of this type, that of β-(N-γ-L-glutamyl)-aminopropionitrile (II) was selected as the first to be tested by synthesis, because a good synthetic method was available,[7] because the $pK$ values of I (2.2 and 9.14) resembled those of glutamic acid, and because II contained the labile γ-glutamyl

(1) Previous paper, THIS JOURNAL, **76**, 2848 (1954).

(2) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by grants from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

(3) H. P. Dupuy and J. G. Lee, *J. Am. Pharm. Assoc. Sci. Ed.*, **43**, 61 (1954).

(4) G. F. McKay, J. J. Lalich, E. D. Schilling and F. M. Strong, *Arch. Biochem. Biophys.*, **52**, 313 (1954).

(5) E. D. Schilling, *Federation Proc.*, **13**, 290 (1954).

(6) W. Dasler, *Science*, **120**, 307 (1954).

(7) F. E. King and D. A. A. Kidd, *J. Chem. Soc.*, 3315 (1949).

linkage. Lee[8] has reported that the lathyrus factor is unstable in acid solution.

The synthesis of the DL-form of II was readily accomplished by condensation of β-aminopropionitrile[9] with phthaloyl-DL-glutamic acid anhydride and subsequent removal of the phthaloyl substituent with hydrazine. The product was found to be very closely similar to I. Synthesis of the L-compound was therefore carried out through the corresponding L-glutamic anhydride derivative, which was conveniently obtained by the method of Nicholls.[10] The synthetic L-compound showed the same physical constants as natural I, and the identity of the two substances was confirmed by comparison of their infrared spectra, which were alike in all respects. Both the L- and DL-forms of the synthetic product exhibited full lathyrus activity in rats, as did β-aminopropionitrile itself.[11]

## Experimental[12]

**Acid Hydrolysis of Isolated Lathyrus Factor. I.**—The crystalline compound I obtained from *Lathyrus odoratus* as previously described[4] was recrystallized until the m.p. rose to 193–194°,[13] $[\alpha]^{22}D$ +18° (*c* 1, water). One-half gram of I and 5 ml. of 11.7 *N* hydrochloric acid were autoclaved at 15 p.s.i. (120°) in a sealed tube for 8 hours. When the dark brown solution was concentrated to about 2 ml. and cooled, 0.254 g. of a white crystalline solid separated. This product was recrystallized from concentrated hydrochloric acid, using a small amount of Darco G-60 charcoal for decolorization, and gave 0.123 g. of colorless plates, III, m.p. 189–191.5° dec.; $[\alpha]^{18}D$ +28° (*c* 1, 6 *N* hydrochloric acid). A mixed m.p. with I gave a depression of 50°. The substance was dried 4 hours at 100° and 0.5 mm. over $P_2O_5$ for analysis.

*Anal.* Calcd. for $C_5H_{10}O_4NCl$: C, 32.7; H, 5.49; N, 7.63; Cl, 19.3. Found: C, 32.48; H, 5.44; N, 7.87; Cl, 18.70.

Paper chromatograms of III and of the crude hydrolysate were run in butanol:acetic acid,[14] collidine:lutidine,[15] and phenol:water[16] systems, and the $R_F$ values of ninhydrin-developed spots compared with those obtained with known amino acids. The results together with the above analytical values strongly suggested that III was glutamic acid. Microbiological assay[17] of the hydrolysate of I with *Lactobacillus arabinosus* 17-5, which does not respond to D-glutamic acid, showed 69% of the theoretical glutamic acid content. This result and the positive rotation showed that III had the L-configuration common in products of natural origin. In addition, the infrared spectrum was found to be identical with that of L-glutamic acid hydrochloride.

When the filtrate from III was made strongly basic, a distinct amine or ammonia odor became evident. The volatile base was carried by means of a stream of nitrogen into a solution of hydrochloric acid. Evaporation left a white solid, which contained no trace of carbon according to the micro-test of Pepkowitz.[18]

**Isolation of β-Alanine.**—Paper chromatograms of the hydrolysate of I showed two spots, one due to glutamic acid, the other unknown. Comparative chromatograms with most of the common amino acids including alanine failed to show a spot matching the unknown, but when β-alanine was tried close agreement both in $R_F$ value and ninhydrin color (lavender) was evident in the phenol system.[16] Sarcosine gave a distinctly different color (very faint pink) and $R_F$ value.

A 1.0-g. sample of I was hydrolyzed as described above, the glutamic acid hydrochloride removed as completely as possible by concentrating, cooling and filtering, and the final filtrate evaporated to dryness. The residue was taken up in water and re-evaporated several times to remove excess hydrochloric acid. The last traces of glutamic acid were then removed by adjusting the solution to *p*H 3.2 and passing it through a 1.2 × 22 cm. column of a weak base anion-exchange resin[19] in the chloride form. The column was washed with water, and the effluent collected at a flow rate of 0.5 ml. per minute until it became ninhydrin-negative. Test runs showed that glutamic acid was retained by the column under these conditions, but that β-alanine was not. An aliquot of the effluent containing approximately 50 mg. of solids was treated with β-naphthalene sulfochloride as described by Weinstock, *et al.*,[20] and the derivative recrystallized from water. Colorless plates were obtained which melted at 134–136°. A sample similarly prepared from known β-alanine melted at 135–136°, and the mixed melting point was 134–136°.

**Phthaloyl-L-glutamic Acid Anhydride.**—This compound was prepared by way of diethyl phthaloyl-L-glutamate as previously described,[7] or more conveniently by the method of Nicholls[10] as follows. To a solution of 148 g. of phthalic anhydride in 300 ml. of boiling glacial acetic acid was added 148 g. of L-glutamic acid, and the mixture was refluxed for 40 minutes. Undissolved glutamic acid, 42.4 g., was filtered off, and the filtrate stored at 4° overnight. A fine white crystalline precipitate, 50.5 g., m.p. 175–180°, which consisted mainly of phthaloyl-DL-glutamic acid, was removed, and the filtrate again chilled at 4° overnight. This treatment resulted in the formation of a second crystalline precipitate, 43.6 g., m.p. 151–155°, which apparently was the desired L-compound. Recrystallization from hot water gave 41.2 g., m.p. 156–159°, $[\alpha]^{25}D$ −44° (*c* 3, dioxane), $[\alpha]^{20}D$ −27.7° (*c* 1, 0.33 *N* $Na_2CO_3$). Concentration of the mother liquors gave a further 30.6 g. of crude material, m.p. 140–151°. Phthaloyl-L-glutamic acid is reported[7,21] to have m.p. 158–159° or 160–161°, $[\alpha]^{18}D$ −27.4° (*c* 1, 0.33 *N* $Na_2CO_3$), $[\alpha]^{25}D$ −48.3 (*c* 3, dioxane).

The anhydride was obtained by dissolving 40.0 g. of the above acid, m.p. 156–159°, in 110 ml. of warm acetic anhydride, concentrating to a thin sirup under reduced pressure, and adding 2–3 volumes of ether. After chilling several hours at 4°, 34.5 g. of product, m.p. 198–203°, was obtained. A small sample recrystallized from ethyl acetate melted at 203–204°, $[\alpha]^{21}D$ −40° (*c* 3, dioxane). Phthaloyl-L-glutamic anhydride is reported to show m.p. 195–196° dec., $[\alpha]^{22}D$ −43.1° (*c* 3, dioxane).[21]

**β-(N-γ-L-glutamyl)-aminopropionitrile.**—A solution of 34.5 g. of phthaloyl-L-glutamic acid anhydride in 60 ml. of boiling dry dioxane was quickly cooled to 0° and 15.2 g. of β-aminopropionitrile was added. After 30 minutes standing at room temperature the product was precipitated as a gummy pale yellow mass by the addition of 50 ml. of ether. The precipitate was taken up in 100 ml. of 10% sodium carbonate solution, 10 g. of 64% aqueous solution of hydrazine hydrate added, and the reaction mixture stored for 2 days at room temperature. The phthalhydrazide formed was then precipitated by addition of 2 *N* hydrochloric acid, and excess chloride ions removed by shaking with 64 g. of silver oxide. The filtrate was adjusted to *p*H 7 with 2 *N* hydriodic acid freshly distilled from red phosphorus, concentrated to 50 ml. under reduced pressure, and 3 volumes of absolute ethanol added. There was obtained 32.5 g. of pinkish-white material, m.p. 182–185°. Recrystallization of a small portion by dissolving in boiling 25% aqueous ethanol and adding absolute ethanol to incipient turbidity

(8) J. G. Lee, *J. Nutrition*, **40**, 587 (1950).

(9) S. R. Buc, *Org. Syntheses*, **27**, 3 (1947).

(10) R. L. L. Nicholls, McGill University, personal communication.

(11) T. E. Bachhuber, G. F. McKay, J. J. Lalich, E. D. Schilling and F. M. Strong, unpublished work.

(12) Microanalyses were carried out by C. W. Beazley, Micro-Tech Laboratories, Skokie, Illinois. The authors wish to thank S. M. Aronovic for the infrared spectra, which were determined in the Baird Associates, Inc., spectrophotometer.

(13) Dasler,[6] reports m.p. 209–210° for the toxic substance isolated by him from *L. odoratus*. However, in our hands the m.p. of a sample kindly supplied by him was 192–192.5° dec. Since the two substances furthermore gave indistinguishable infrared spectra, there is no doubt that they were identical.

(14) A. J. Woiwod, *J. Gen. Microbiol.*, **3**, 312 (1949).

(15) C. E. Dent, *Biochem. J.*, **43**, 169 (1948).

(16) H. K. Berry and L. Cain, *Arch. Biochem.*, **24**, 179 (1949).

(17) A. M. Violante, R. J. Sirny and C. A. Elvehjem, *J. Nutrition*, **47**, 307 (1952). The authors wish to thank E. W. Lewis and F. N. Hepburn for carrying out this assay.

(18) L. P. Pepkowitz, *Anal. Chem.*, **23**, 1716 (1951).

(19) Amberlite IR4B, Rohm and Haas Company.

(20) H. H. Weinstock, H. K. Mitchell, E. F. Pratt and R. J. Williams, THIS JOURNAL, **61**, 1421 (1939).

(21) J. W. Clark-Lewis and J. S. Fruton, *J. Biol. Chem.*, **207**, 477 (1954).

gave fine white needles, m.p. 193–194° dec., $[\alpha]^{20}D$ +18° (*c* 1, water). Mixed with natural I, the m.p. was 193–194° dec. The infrared spectra of both natural and synthetic I were measured as micro-mulls in purified mineral oil and no significant differences were noted.

The DL-form of I was prepared in the same manner, starting with phthaloyl-DL-glutamic acid anhydride. The product was obtained in the form of glistening colorless platelets, m.p. 191–192° dec.[22]

*Anal.* Calcd. for $C_8H_{13}O_3N_3$: N, 21.10. Found: N, 21.04, 21.22.

(22) Bath preheated to 180°.

MADISON, WISCONSIN

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[CONTRIBUTION NO. 142 FROM THE GENERAL LABORATORIES OF THE UNITED STATES RUBBER COMPANY]

# Some *meso*-Substituted Anthracenes. I. 9,10-Bis-(chloromethyl)-anthracene as a Synthetic Intermediate[1]

BY MAX W. MILLER, ROGER W. AMIDON AND PLINY O. TAWNEY

RECEIVED OCTOBER 21, 1954

An improved procedure for the preparation of 9,10-bis-(chloromethyl)-anthracene (I) is reported. Compounds prepared by displacement reactions from I are described as well as certain more remote derivatives.

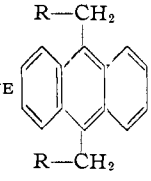
The chloromethylation of anthracene has been developed so that good yields of 9,10-bis-(chloromethyl)-anthracene (I) can be obtained conveniently. This has permitted the synthesis of a new series of *meso*-substituted anthracenes by displacement reactions from I and by further transformations. For example, it has been possible to prepare for the first time 9,10-anthracenediacetic acid XVI although the lower and higher homologs have long been known.

Ethers VIII, IX derived from I are, like benzyl ethers, sensitive toward acids. Because they are more soluble and less irritating, they can profitably be substituted for I in certain acidic reactions.

Previous attempts to prepare the diamine II by inconvenient routes[5,6] were not successful, and the correct analysis and physical properties have never been reported. It is possible to prepare this amine in good yields merely by enclosing I with anhydrous ammonia in a rocked autoclave, a method similar to

TABLE I

COMPOUNDS DERIVED FROM 9,10-BIS-(CHLOROMETHYL)-ANTHRACENE



| | R = | | | | | | |
|---|---|---|---|---|---|---|---|
| I | Cl | VII | $OCOCH_3$ | XIII | $SCH_2CH_2CN$ | XIX | $CH_2COOH$ |
| II | $NH_2$ | VIII | $OCH_3$ | XIV | CN | XX | $CH_2COOCH_3$ |
| III | $NH_2 \cdot HCl$ | IX | $OC_2H_5$ | XV | $CONH_2$ | XXI | $CH_2CONH_2$ |
| IV | $NHCOCH_3$ | X | SH | XVI | COOH | XXII | $CH_2CH_2OH$ |
| V | NCO | XI | $SCOCH_3$ | XVII | $COOCH_3$ | | |
| VI | OH | XII | $SCH_2COOCH_3$ | XVIII | $CH_2OH$ | | |

All of the compounds listed in Table I are intrinsically yellow although anthracene is colorless except for a blue-violet fluorescence. Solubilities vary widely with substituent groups. Thus 9,10-anthracenedimethanethiol (X) is quite soluble in common organic solvents, while 9,10-anthracenedimethanol (VI) dissolves only in certain high-boiling, polar solvents such as nitrobenzene or anisole.

The parent compound I of this series has been known for some time,[2–4] but a satisfactory and detailed procedure for its preparation has never been published. It is crystalline, melts with decomposition and is slightly soluble in most organic solvents but recrystallizable from toluene or dioxane. It is a skin irritant capable of causing acute sensitization, and should be handled with care.

(1) Part of this work was done under contract NOBS-55413 with the Bureau of Ships, Department of the Navy.

(2) I. G. Farbenindustrie, German Patent 533,850; *Chem. Zentr.*, **104**, I, 2396 (1931).

(3) I, Ia. Postovskii and N. P. Bednyagina, *J. Gen. Chem. U.S.S.R.*, **7**, 2919 (1937).

(4) G. M. Badger and J. W. Cook, *J. Chem. Soc.*, 802 (1939).

that mentioned for the conversion of 9-chloromethylphenanthrene to the corresponding amine.[7] Treatment of the stable dihydrochloride III with phosgene gives 9,10-anthracenedimethyl isocyanate (V).

While it may be possible to convert I directly to 9,10-anthracenedimethanol (VI), the glycol can be prepared in excellent yields through the diacetate. This diacetate was prepared either by treatment of I with potassium acetate,[4] or by treating the ether IX with acetic acid and a trace of mineral acid.

The dithiol X was prepared through the diisothiouronium chloride. An earlier attempt to prepare this dithiol gave material, m.p. 145° (s.130°) with no analysis reported.[8] The present procedure leads to good yields of X, m.p. 217–218° (s.216°), with correct analysis and suitable derivatives.

(5) P. De Bruyn, *Compt. rend.*, **231**, 295 (1950).

(6) W. Herzberg and H. Lange, German Patent 442,774.

(7) J. von Braun, *Ber.*, **70B**, 979 (1937).

(8) P. O. Tawney, U. S. Patent 2,583,975.