zimidazole showed no depression in melting point. The infrared spectrum of this product proved to be identical with the infrared spectrum of 2-methylbenzimidazole.

8. Reaction of 3-(2-Benzimidazolyl)-1,1,1-trifluoro-2-propanone with Sodium Ethoxide.—A solution of 0.19 g. (0.0086 mole) of sodium metal in 20 ml. of absolute ethanol was mixed with 1 g. (0.0043 mole) of 3-(2-benzimidazolyl)-1,1,1-trifluoro-2-propanone, and the mixture was heated under reflux for 30 minutes. After the resulting solution was cooled, concentrated hydrochloric acid was added dropwise until the neutral point was reached. The sodium chloride which formed was removed from the precipitate by extracting with 100 ml. of water. The product was then removed by filtration and dried, m.p. 270° dec. A mixture of this product with the starting material showed no depression in melting point.

9. Methylation of 3-(2-Benzimidazolyl)-1,1,1-trifluoro-2-propanone. Preparation of 3-(1-Methyl-2-benzimidazolyl)-1,1,1-trifluoro-2-hydroxy-2-butene (IV).—A solution of 0.19 g. (0.0086 mole) of sodium in 20 ml. of absolute ethanol was mixed with 1 g. (0.0043 mole) of 3-(2-benzimidazolyl)-1,1,1-trifluoro-2-propanone, and the mixture was heated in a water-bath at 50° for 30 minutes; 4 ml. (0.06 mole) of methyl iodide was added gradually to the solution with shaking during the heating period. After the addition of methyl iodide was completed, the solution was heated for an additional 15 minutes. The ethanol and excess methyl iodide were removed by distillation, and the residue was treated with 100 ml. of cold water. The product which formed was collected by filtration and recrystallized from 50% aqueous ethanol, yield 91%, m.p. 178° dec.

Anal. Calcd. for $C_{12}H_{11}N_2F_3O$: C, 56.24; H, 4.32; N, 10.93. Found: C, 56.04; H, 4.21; N, 11.06.

10. Determination of the Enolic Content of 3-(2-Benzimidazolyl)-1,1,1-2-propanone.—A solution of 0.001 mole of 3-(2-benzimidazolyl)-1,1,1-trifluoro-2-propanone in 50 ml. of absolute methanol was cooled to −5°, and an excess of approximately 0.1 *N* bromine in absolute methanol was added with stirring. A slight excess of di-isobutylene was added until the red color of the bromine disappeared. The time consumed in adding the bromine and decolorizing the solution was approximately 15 seconds. Then, 5 ml. of 10% aqueous potassium iodide was added and the mixture warmed to 30° by dipping the flask in hot water. The solution was allowed to stand for 10 minutes to make sure that the reaction was completed. The solution was titrated with stirring, with standard 0.1 *N* sodium thiosulfate solution until the color became light yellow. At this point 200 ml. of water and 5 ml. of starch solution were added, and the titration continued until the blue color which formed disappeared.

The exact amounts of the compounds used and the results obtained are

Sample, g.	Ml. of 0.1 *N* bromine soln.	Ml. of 0.1 *N* thiosulfate soln.	Enol, % present
0.2286	25	16.54	69.93
.2288	25	16.92	68.49
.2284	25	16.52	69.93

11. Qualitative Tests for the Structure Analysis of 4-(Trifluoromethyl)-1H-1,5-benzodiazepin-2(3H)-one, 3-(*o*-Aminophenylimino)-butyrohydroxamic Acid and 3-(2-Benzimidazolyl)-1,1,1-trifluoro-2-propanone.—The qualitative tests for structure analysis were carried out according to the procedures described by Shriner, Fuson and Curtin[8] unless otherwise noted.

(8) R. L. Shriner, R. C. Fuson and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th ed., John Wiley and Sons, Inc., New York, N. Y., 1956.

PHILADELPHIA 4, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FACULTY OF ENGINEERING, UNIVERSITY OF TOKUSHIMA]

The Chemistry of Antimycin A. VII. Synthesis of Antimycic Acid and its Analogs

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RECEIVED MARCH 19, 1959

N-(3-Aminosalicyloyl)-L-threonine (natural antimycic acid) (VIb) has been prepared from 3-aminosalicylic acid benzyl ether (I). The synthesis of N-(3-aminosalicyloyl)-DL-threonine (VIa) also has been achieved by the same method.

In a previous paper[1] it was reported that the synthesis of L- or DL-antimycic acid-methyl ester-methyl ether was successfully achieved by condensation of nitrosalicylic acid methyl ether with L- or DL-threonine and that the synthetic L-peptide was identical with natural antimycic acid-methyl ester-methyl ether.

More recently we have found that 3-nitrosalicylic acid benzyl ether[2] (I) can be prepared in good yield by benzylation of 3-nitrosalicylic acid methyl ester[3] in dimethylformamide solution.

The synthesis of natural antimycic acid from 3-nitrosalicylic acid benzyl ether (I) has been carried through by almost the same procedure described in the preceding paper[1] as shown in Fig. 1.

The benzyl ether I was converted into the corresponding acid chloride II by heating with thionyl chloride, and this product was condensed with DL-threonine to give N-(3-nitro-2-benzyloxybenzoyl)-DL-threonine (IIIa). Compound IIIa was converted into N-(3-nitro-2-benzyloxy-benzoyl)-α-aminocrotonic azlactone (IV) by heating with acetic anhydride and pyridine. Compound IIIa also was converted easily into colorless plates of N-(3-aminosalicyloyl)-DL-threonine (DL-antimycic acid)(VIa) by catalytic reduction with palladium-on-charcoal in methanol solution. The infrared spectrum of this compound (VIa) was nearly identical with that of natural antimycic acid.[4] Furthermore VIa could be converted with diazomethane into a methyl ester-methyl ether VIIa identical with the sample previously synthesized.[1]

For the synthesis of natural antimycic acid N-(3-nitro-2-benzyloxybenzoyl)-L-threonine (IIIb) was obtained by condensation of the acid chloride II with L-threonine. Recrystallization from benzene gave long needles of a solvated product containing one mole of benzene. Compound IIIb gave the same azlactone IV as that derived from the DL-threonine peptide (IIIa) by the usual method. L-Antimycic acid (N-(3-aminosalicyloyl)-L-threonine) (VIb) was obtained from

(1) F. S. Okumura, M. Masumura, T. Horie and F. M. Strong, THIS JOURNAL, **81**, 3753 (1959).

(2) The syntheses of nitrosalicyclic acid benzyl ether, N-(substituted-2-benzyloxybenzoyl)-glycine and -leucine have been reported by W. O. Foye and R. L. Hull, *J. Amer. Pharm. Assoc.*, **42**, 50 (1953).

(3) Prepared by esterification of 3-nitrosalicylic acid, which was obtained from Eastman Organic Chemicals.

(4) G. M. Tener, F. M. Bumpus, B. R. Dunshee and F. M. Strong, THIS JOURNAL, **75**, 1100 (1953).

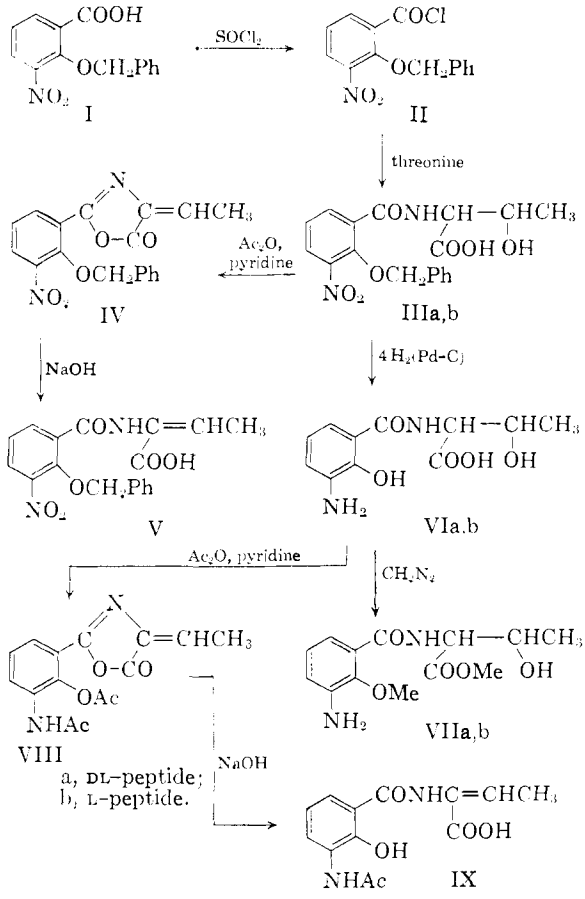

Fig. 1.

IIIb as plate-like crystals by catalytic reduction with palladium-on-charcoal in methanol solution. The L-antimycic acid so prepared had the same melting point as the natural material[4] and the mixed melting point showed no depression. Furthermore the infrared absorption spectra of both substances were identical. Methylation of VIb with diazomethane yielded L-antimycic acid-methyl ester-methyl ether identical with that prepared from 3-nitrosalicylic acid methyl ether by the method described in the preceding paper.[1]

Both L- and DL-antimycic acid (VIb and VIa) showed the same behavior toward acetic anhydride and pyridine and gave the same N-(3-acetaminoacetylsalicyloyl)-α-aminocrotonic azlactone[4] (VIII). The product had the same melting point as the azlactone prepared from natural antimycic acid, and the mixed melting point showed no depression. Hydrolysis of VIII with 0.1 *N* aqueous sodium hydroxide yielded N-(3-acetaminosalicyloyl)-α-aminocrotonic acid (IX).

Experimental

3-Nitrosalicylic Acid Benzyl Ether (I).—Ten grams of 3-nitrosalicylic acid methyl ester[3] and 16 g. of benzyl chloride were dissolved in 80 g. of warm dimethylformamide (50–60°), and sodium ethoxide solution (1.3 g. of sodium in 15 ml. of ethanol) was added with shaking. The mixture was refluxed for 2 hours until the color of the solution turned from orange to yellow. Then 5 g. of benzyl chloride and additional sodium ethoxide solution (0.6 g. of sodium in 8 ml. of ethanol) were added and heating was continued. After the reaction appeared to be complete, the precipitated sodium chloride was filtered off and the filtrate was concentrated under reduced pressure. The residue was taken up in ether and washed well with dilute aqueous sodium hydroxide solution and with water. After distilling off the ether, the residual oily substance was dissolved in ethanol and hydrolyzed with 20% aqueous sodium hydroxide solution. The reaction mixture was washed with ether, acidified with hydrochloric acid and then allowed to stand in an ice-box to crystallize. The product was filtered off, triturated with benzene, and refiltered to yield 6 g. (43%), and an additional 2 g. was obtained from the mother liquor to make a total yield of 8 g. (58%). This crude product was purified by recrystallization from benzene giving pale yellow needles, m.p. 129–130°.

Anal. Calcd. for $C_{14}H_{11}O_5N$: N, 5.13. Found: N, 5.23.

N-(3-Nitro-2-benzyloxybenzoyl)-DL-threonine (IIIa).— A mixture of 2.7 g. of 3-nitrosalicylic acid benzyl ether (I) and 12 g. of thionyl chloride was heated for 30 minutes at 60–65° and then the excess of thionyl chloride was removed under reduced pressure. The residue II was dissolved in 20 ml. of absolute tetrahydrofuran and this solution was added in portions of 1 to 2 ml. at intervals of 3 to 5 minutes, to a DL-threonine solution (1.5 g. of DL-threonine in 10 ml. of *N* sodium hydroxide solution) with vigorous stirring and ice cooling. During the reaction period, the solution was kept alkaline to thymol blue with 2 *N* sodium hydroxide solution. After the addition of acid chloride, the stirring was continued for another hour. The reaction mixture was washed with ether, acidified with hydrochloric acid, and then extracted with ether. The extract was concentrated, after washing with water. The residue was dissolved in 10–20 ml. of benzene and allowed to stand in an ice-box to crystallize. The yield after filtering and washing with benzene was 2.2 g. (60%). Recrystallization from 50% aqueous ethanol gave colorless needles, m.p. 141–142°.

Anal. Calcd. for $C_{18}H_{18}O_7N_2$: N, 7.49. Found: N, 7.50.

N-(3-Nitro-2-benzyloxybenzoyl)-L-threonine (IIIb) was synthesized from 3 g. of 3-nitrosalicylic acid benzyl ether (I) and 1.55 g. of L-threonine by the above-described procedure. The yield was 2.8 g. (57%). After recrystallization from benzene pale yellow needles, m.p. 75–85°, $[\alpha]^{17}D$ −12° (*c* 1.50, methanol), were obtained.

Anal. Calcd. for $C_{18}H_{18}O_7N_2 + C_6H_6$: C, 63.72; H, 5.31; N, 6.19. Found: C, 63.49; H, 5.17; N, 6.22, 6.10.

N-(3-Nitro-2-benzyloxybenzoyl)-α-Aminocrotonic Azlactone (IV). (a) **From N-(3-Nitro-2-benzyloxybenzoyl)-DL-threonine** (IIIa).—A mixture of 100 mg. of IIIa, 0.3 ml. of acetic anhydride and 2 drops of pyridine was heated for 3 minutes at 95–100°. After cooling, the mixture was added to 1 ml. of water and allowed to stand in an ice-box to crystallize (yield 85 mg., 94%). The product was recrystallized from acetic acid or ethanol to yield pale yellow needles melting at 130–131°.

Anal. Calcd for $C_{18}H_{14}O_5N_2$: N, 8.28. Found: N, 8.18.

(b) **From N-(3-Nitro-2-benzyloxybenzoyl)-L-threonine** (IIIb).—The azlactone IV was obtained from 100 mg. of IIIb in 87% (65 mg.) yield by the above method. After recrystallization from ethanol, the product showed the same melting point as the above and the mixed melting point showed no depression.

Anal. Calcd. for $C_{18}H_{14}O_5N_2$: N, 8.28. Found: N, 8.35.

N-(3-Nitro-2-benzyloxybenzoyl)-α-aminocrotonic Acid (V).—A mixture of 105 mg. of N-(3-nitro-2-benzyloxybenzoyl)-α-aminocrotonic azlactone (IV), 6 ml. of 0.2 *N* aqueous sodium hydroxide and 6 ml. of ethanol was stirred at room temperature (20–30°) until the solution became clear. After filtering, the solution was acidified with hydrochloric acid and then allowed to stand overnight. The yield after filtering and washing with ether was 70 mg. (63%). Recrystallization from ethanol gave colorless needles, m.p. 193–194°.

Anal. Calcd. for $C_{18}H_{16}O_6N_2$: N, 7.87. Found: N, 7.77.

N-(3-Aminosalicyloyl)-DL-threonine (DL-Antimycic Acid) (VIa).—To 60–70 ml. of methanol solution containing 0.65 g. of N-(3-nitro-2-benzyloxybenzoyl)-DL-threonine (IIIa) was added 0.48 g. of palladium-on-charcoal (5%) and the

mixture was shaken in a hydrogen atmosphere for 5–15 minutes. The calculated amount of hydrogen had then been absorbed. The catalyst was filtered off, the filtrate concentrated to about 1 ml. at reduced pressure, and the concentrate which contains large amount of crystals allowed to stand in an ice-box to complete crystallization. The yield after filtering and washing with methanol was 0.39 g. (91%). The product was purified by recrystallization from 20–40% aqueous ethanol and was obtained as colorless plates, m.p. 221.5–222.5° dec. For analysis the crystals were dried at 0.1–0.2 mm. and 100° for one hour.

Anal. Calcd. for $C_{11}H_{14}O_5N_2$: C, 51.97; H, 5.51; N, 11.03. Found: C, 51.80; H, 5.67; N, 11.06.

N-(3-Aminosalicyloyl)-L-threonine (L-Antimycic Acid) (VIb).—An amount of 1.1 g. of N-(3-nitro-2-benzyloxybenzoyl)-L-threonine (IIIb) was reduced catalytically with 0.7 g. of palladium-on-charcoal (5%) in 70 ml. of methanol solution by the above method, and the product was obtained in 84% (0.52 g.) yield. Recrystallization from 20–40% aqueous ethanol gave colorless plates, m.p. 224–225° dec., $[\alpha]^{17}D$ + 15° (*c* 1.36, 3% hydrochloric acid). The mixed melting point of this product with natural antimycic acid[4] showed no depression. For analysis the crystals were dried at 0.1–0.2 mm. and 100° for one hour.

Anal. Calcd. for $C_{11}H_{14}O_5N_2$: C, 51.97; H, 5.51; N, 11.03. Found: C, 51.67; H, 5.69; N, 11.17.

N-(3-Aminosalicyloyl)-DL-threonine-Methyl Ester-Methyl Ether (DL-Antimycic Acid-Methyl Ester-Methyl Ether) (VIIa).—When 104 mg. of N-(3-aminosalicyloyl)-DL-threonine (VIa) was methylated with diazomethane by the procedure used for preparation of natural antimycic acid-methyl ester-methyl ether,[1] the yield was 40 mg. (36%). Recrystallization from ethyl acetate gave colorless needles, m.p. 127.5–129°. For analysis the crystal were dried in a vacuum at 80° for one hour. This product did not depress the melting point of N-(3-amino-2-methoxybenzoyl)-DL-threonine-methyl ester prepared from N-(3-amino-2-methoxybenzoyl)-DL-threonine.[1]

Anal. Calcd. for $C_{13}H_{18}O_5N_2$: N, 9.93. Found: N, 9.86.

N-(3-Aminosalicyloyl)-L-threonine-Methyl Ester-Methyl Ether (L-Antimycic Acid-Methyl Ester-Methyl Ether) (VIIb).—A yield of 50 mg. (43%) of VIIb was obtained by the above method from 100 mg. of N-(3-aminosalicyloyl)-L-threonine (VIb). Recrystallization from ethyl acetate gave colorless needles of m.p. 155–156°, which were identical with natural antimycic acid-methyl ester-methyl ether. For analysis the crystals were dried in a vacuum at 80° for one hour.

Anal. Calcd. for $C_{13}H_{18}O_5N_2$: N, 9.93. Found: N, 10.02.

N-(3-Acetaminoacetylsalicyloyl)-α-aminocrotonic Azlactone (Antimycic Acid Diacetate) (VIII).—(a) A mixture of 45 mg. of N-(3-aminosalicyloyl)-DL-threonine (VIa), 0.7 ml. of acetic anhydride and 2 drops of pyridine was heated for 3 minutes at 95–100°. After cooling, the crystals were filtered off and washed with acetic anhydride (yield 43 mg., 80%). Recrystallization from acetic anhydride gave fibrous needles, m.p. 210–211° dec.

Anal. Calcd. for $C_{15}H_{14}O_5N_2$: N, 9.27. Found: N, 9.48.

(b) Reaction of 80 mg. of N-(3-aminosalicyloyl)-L-threonine (VIb) with 1.5 ml. of acetic anhydride and 3 drops of pyridine as above gave the same azlactone (yield 86 mg., 90%). This product upon recrystallization from acetic anhydride gave fibrous needles, m.p. 210–211° dec. The mixed melting point with natural antimycic acid diacetate[4,5] showed no depression.

Anal. Calcd. for $C_{15}H_{14}O_5N_2$: N, 9.27. Found: N, 9.22.

N-(3-Acetaminosalicyloyl)-α-aminocrotonic Acid (IX).—A mixture of 85 mg. of N-(3-acetaminoacetylsalicyloyl)-α-aminocrotonic azlactone (VIII) and 7 ml. of 0.1 *N* aqueous sodium hydroxide was stirred at room temperature (15–20°) until the solution became clear. When the solution was adjusted to about *p*H 1 with hydrochloric acid, colorless plates were deposited. The yield after filtering and washing with water was 73 mg. (93%). Recrystallization from 50% aqueous ethanol or ethanol gave colorless plates or fine needles, m.p. 220–221° dec. For analysis the crystals were dried at 0.1–0.2 mm. and 100° for one hour.

Anal. Calcd. for $C_{13}H_{14}O_5N_2$: N, 10.07. Found: N, 10.20.

Acknowledgments.—The authors wish to express their sincere appreciation to Prof. F. M. Strong of the University of Wisconsin for his interest, and his helpful comments made in connection with the preparation of this manuscript; and to the Tanabe Pharmaceutical Company for its support. Thanks are due to Miss Teruko Ueda of this Laboratory for the microanalyses.

(5) Natural antimycic acid diacetate described by Tener, *et al.*,[4] melted at 202–202.5°, but after recrystallization from acetic anhydride the melting point was raised to 210–211°.

TOKUSHIMA, JAPAN

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF BOSTON UNIVERSITY]

Location of Ketone and Hydroxyl Functions of Cassaic Acid[1]

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RECEIVED FEBRUARY 12, 1959

Methyllithium reacts with the acetyl derivative of decarboxylated cassaic acid to furnish a product that can be aromatized to 1,7,8,9-tetramethylphenanthrene. Methylmagnesium iodide adds to only one of the two ketone groups in decarboxylated diketocassenic acid. The addition product after lithium aluminum hydride reduction and selenium aromatization is converted to 1,2,7,8-tetramethylphenanthrene. The work establishes the correctness of the earlier, provisionally assigned, positions for the hydroxyl and the ketone groups of cassaic acid.

Formulation I, advanced by Humber and Taylor[2a] and by Tondeur,[2b] is consonant with the observed chemistry of cassaic acid.[3] Although the carbon skeleton has been established,[4] the locations of the hydroxyl and ketone groups have remained unproved. We are now reporting evidence in support of the provisional assignments of position 2 for the hydroxyl and position 9 for the ketone group. Our plan of attack, as discussed below, called for the use of methyl groups as markers for the oxygen functions.

(1) Abstracted from the Dissertation submitted by Gwendolyn M. Sherman to the Graduate School of Boston University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, 1959.

(2) (a) L. G. Humber and W. I. Taylor, *J. Chem. Soc.*, 1044 (1955); (b) R. Tondeur, Ph.D. Thesis, E.T.H., Zurich, 1950.

(3) *Cf.* G. Dalma in Chapter 36 of "The Alkaloids," by R. H. F. Manske and H. L. Holmes, Vol. IV, Academic Press, Inc., New York, N. Y., 1954; T. A. Henry, "The Plant Alkaloids," 4th edition, Blakiston Co., Philadelphia, Pa., 1949, p. 725.

(4) See F. E. King, T. J. King and J. M. Uprichard, *J. Chem. Soc.*, 3428 (1958).