Attempted Deuteration of 4a-Methyl-1,3,9-triphenyl-4a*H*-fluorene in Deuterioacetic Acid–Concentrated Sulfuric Acid.—Two grams (0.005 mole) of Ia was added to a solution of 50 g. of deuterioacetic acid and 100 mg. of concentrated sulfuric acid (0.001 mole). Immediately upon addition of the hydrocarbon the solution became deep blue-green in color. The solution was refluxed with stirring for 2 hr. and then allowed to stand at room temperature for 12 hr. Twelve grams of 99.5% deuterium oxide was then added to bring about precipitation of Ia. The solid was collected by filtration. The infrared and nuclear magnetic resonance spectra showed no incorporation of deuterium.

Attempted Deuteration of 4a-Methyl-1,3,9-triphenyl-4a*H*-fluorene, Ia, in 50% Deuteriotrifluoroacetic Acid–Deuterium Oxide.—Two grams (0.005 moles) of Ia was added to 70 g. of 50% (by weight) deuteriotrifluoroacetic acid–99.5% deuterium oxide. This blue-green heterogeneous mixture was refluxed for 4 days. The reaction mixture was then cooled and the solid, Ia, collected by filtration. The infrared and n.m.r. spectra showed no incorporation of deuterium.

Bromination of 4a-Methyl-1,3,9-triphenyl-4a*H*-fluorene with Ethyl Bromide in Dimethyl Sulfoxide. Preparation of 4-Bromo-4a-methyl-1,3,9-triphenyl-4a*H*-fluorene, Ic.—Two grams (0.005 moles) of Ia was added to 100 ml. of dry dimethyl sulfoxide in a 250-ml. three-necked, round-bottom flask equipped with a thermometer, calcium chloride protected condenser and an automatic stirrer. Ten milliliters of ethyl bromide was then added to the reaction mixture. The mixture was vigorously stirred while it was slowly heated. At a temperature of 50° the color of the reaction mixture became deep blue-green and at 60° complete solution occurred. The solution was heated to 70°. Approximately 15 min. after the color change to blue-green there was another color change from blue-green to yellow. The solution was allowed to cool to room temperature after the second color change. Two hundred milliliters of water was added to the reaction solution, causing the precipitation of a bright yellow solid. This solid was collected by filtration, giving 2.3 g. Recrystallization twice from a 1:1 mixture of ethanol and chloroform gave 1.6 g. (66%) of yellow crystalline 4-bromo-4a-methyl-1,3,9-triphenyl-4a*H*-fluorene, Ic, m.p., 212–214°.

Anal. Calcd. for $C_{32}H_{23}Br$: C, 78.85; H, 4.72. Found: C, 78.66; H, 4.73.

Bromine was detected by sodium fusion and by the Beilstein test. The infrared spectrum of Ic was very similar to that of Ia. The n.m.r. spectrum of Ic in chloroform showed, in addition to aromatic and methyl absorption, only one vinyl proton at τ, 3.70. The n.m.r. spectrum of the starting material, Ia, showed absorption of two vinyl protons at τ, 3.33.

Reaction of 4-Bromo-4a-methyl-1,3,9-triphenyl-4a*H*-fluorene with Butyllithium Followed by Deuterium Oxide. Preparation of 4a-Methyl-1,3,9-triphenyl-4a*H*-fluorene-4d.—One and one half grams (0.0031 mole) of Ia was dissolved in 200 ml. of anhydrous diethyl ether in a 500-ml. three-necked, round-bottom flask equipped with a dropping funnel and a calcium chloride drying tube. Twenty milliliters of a butyllithium solution (15% by weight in one third pentane and two thirds heptane, Foote Mineral Company) was added in one portion. Immediately upon addition of the butyllithium, the solution became deep blue-green. This solution was allowed to stand at room temperature for 15 min. Four grams of 99.5% deuterium oxide was then slowly added causing an immediate vigorous reaction. The color changed from deep blue-green to yellow. The solvent was removed by distillation under reduced pressure leaving a yellow solid. This material was recrystallized from a 1:1 mixture of ethanol and ether, giving 0.8 g. (0.0019 mole, 61%) of 4a-methyl-1,3,9-triphenyl-4a*H*-fluorene-4d, m.p. 179–180°.

The infrared spectrum was identical to that of Ia except for a small peak at 2250 cm.$^{-1}$ due to the C—D stretching frequency. The n.m.r. spectrum showed absorption of only one vinyl proton at τ, 3.40. The n.mr. spectrum of Ia showed absorption of two vinyl protons at τ, 3.33.

N.m.r. Spectroscopy.—The n.m.r. spectra were recorded by Mr. D. Johnson and his associates with a Varian Associates high resolution spectrometer (A–60) at a frequency of 60 Mc. per second. Spectra were obtained in 30% solutions with tetramethylsilane as an internal standard. Chemical shifts are expressed as shielding values, τ, as defined by G. V. D. Tiers.[16]

(16) G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

The Chemistry of Actinamine[1]

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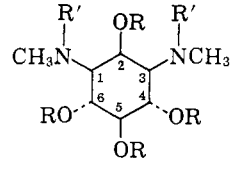
Chemical evidence is presented for the structure and stereochemistry of actinamine, I, a degradation product of the antibiotic actinospectacin.

Actinospectacin,[3] a broad spectrum antibiotic produced by the actinomycete *Streptomyces spectabilis* has the composition $C_{14}H_{24}N_2O_7 \cdot 2HCl \cdot 5H_2O$. Hydrolysis of actinospectacin with hot 6 *N* hydrochloric acid gives a crystalline fragment, actinamine dihydrochloride $C_8H_{18}N_2O_4 \cdot 2HCl$. The free base, actinamine $C_8H_{18}N_2O_4 \cdot 1/2H_2O$, is readily obtained from its dihydrochloride by ion exchange. In the following discussion we present chemical evidence for the structure and stereochemistry of actinamine (I).



I. R = R′ = H
II. R = R′ = CH_3CO
III. R = H, R′ = CH_3CO
VI. R = H, R′ = CH_3
VII. R = CH_3CO, R′ = CH_3
XI. R = H, R′ = $COOCH_3$
XII. R = CH_3CO, R′ = $COOCH_3$

Functional Groups and Carbon Skeleton of Actinamine.—The infrared absorption spectrum of actinamine shows no evidence of unsaturation. Strong absorption at 3300 cm.$^{-1}$ shows the presence of hydroxyl and/or amino groups. The basic nature of both nitrogen atoms is obvious from the formation of a dihydrochloride. Potentiometric titration of the dihydrochloride against sodium hydroxide gave two breaks in the curve and the neutralization equivalent obtained agreed with that expected for I. Titration of actinamine against aqueous hydrochloric acid gave pK_a values 7.2

(1) (a) Research supported by grant E-1138 of the U. S. Public Health Service; (b) A. L. Johnson, Union Carbide Predoctoral Fellow at the University of Rochester, 1962–1963.

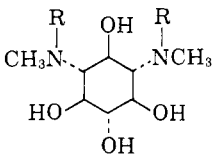
(2) To whom inquiries should be addressed.

(3) (a) D. J. Mason, A. Dietz, and R. M. Smith, *Antibiot. Chemotherapy*, **11**, 118 (1961); (b) M. E. Bergy, T. E. Eble, and R. R. Herr, *ibid.*, **11**, 661 (1961); (c) P. F. Wiley, *J. Am. Chem. Soc.*, **84**, 1514 (1962); (d) D. D. Chapman, R. L. Autrey, R. H. Gourlay, A. L. Johnson, J. Souto, and D. S. Tarbell, *Proc. Natl. Acad. Sci., U.S.*, **48**, 1108 (1962); (e) After the preparation of this manuscript a communication by H. Hoeksema, A. D. Argoudelis, and P. F. Wiley describing the complete structure of actinospectacin appeared in *J. Am. Chem. Soc.*, **84**, 3212 (1962).

and 9.4.[4] Actinamine gives negative reactions towards Tollens reagent, Benedicts reagent, and the iodoform test. It reduces hot acidified potassium dichromate or potassium permanganate slowly, and resembles streptamine[5] in its resistance to reduction by hydriodic acid and red phosphorus.

The cyclic nature of actinamine is confirmed by its molecular formula and the products of periodate oxidation. At pH 4.6 and room temperature one mole of actinamine consumes 6.1 moles of periodate and releases 1.95 moles of methylamine. Both nitrogen atoms must be present as methylamino groups. No formaldehyde[6] was detected in the reaction mixture, excluding the presence of a primary alcohol grouping. The formation of a hexaacetyl derivative (II), $C_{20}H_{30}N_2O_{10}$, and the ready O-deacetylation of this compound to the N,N′-diacetyl derivative (III), $C_{12}H_{22}N_2O_6$, indicate clearly that actinamine possesses four secondary hydroxyl groups. Neither II nor III possesses any titratable basic groups.[4] Compound II shows no hydroxyl absorption in the 3100–3700-cm.$^{-1}$ region of the infrared, but ester carbonyl functions are identified at 1740 cm.$^{-1}$, amide carbonyl functions at 1640 cm.$^{-1}$, and acetate groups at 1250 cm.$^{-1}$. Compound III shows hydroxyl absorption at 3170 cm.$^{-1}$ and amide carbonyl absorption at 1625 cm.$^{-1}$. At pH 4.6 and room temperature one mole of III consumes 2.26 moles of periodate and liberates 1.25 moles of formic acid[7] over a period of twenty hours.

Stereochemistry of Actinamine.—Compounds I, II, III, and actinamine dihydrochloride are optically inactive over the wave length range 280 to 600 mμ.[8] There are eight possible *meso* forms for actinamine. The all *trans* isomer IV has been eliminated by Wiley[3c]

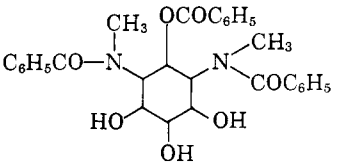


IV. R = H
V. R = CH_3

who showed that N,N′-dimethylactinamine (VI) was not identical with N,N′-tetramethylstreptamine (V). Nuclear magnetic resonance studies on VI[9] and N,N′-dimethyl-O-tetraacetylactinamine (VII)[10] have been presented to establish the stereochemistry of actinamine as I. We present the following chemical evidence in support of this stereochemistry.

The strong band near 1050 cm.$^{-1}$ in the infrared spectrum of inosamines is shifted slightly towards lower frequencies when the amino group is *cis* to an adjacent hydroxyl group.[11] Absorption at 1045 cm.$^{-1}$ by actinamine is in the expected position for one hydroxyl group *cis* to an adjacent amino group.

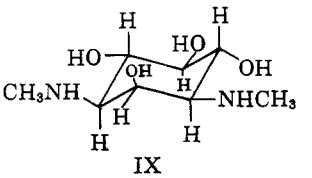
Schotten–Baumann benzoylation of actinamine produces a polybenzoate which can be partially debenzoylated to N,N′,O-tribenzoylactinamine (VIII), $C_{29}H_{30}N_2O_7$. Structure VIII is confirmed by infrared



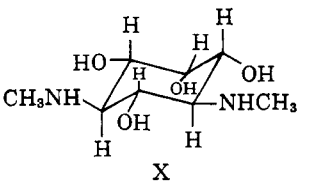
VIII

absorption at 3550 cm.$^{-1}$ (hydroxyl), 1720 cm.$^{-1}$ (ester carbonyl), 1625 cm.$^{-1}$ (amide carbonyl), and 1260 cm.$^{-1}$ (benzoate ester) and by the consumption of 1.57 moles of periodate over four days at room temperature. The periodate cleavage and the ready formation of VIII under mild conditions suggest that the hydroxyl groups have a *trans* diequatorial relationship.[12] Since I is a *meso* form the adjacent hydroxyl groups on C-4, C-5, and C-6 should have a *trans* equatorial relationship.

The n.m.r. spectrum[13] of I in deuterium oxide can be explained only by the two possibilities IX and X. The six equivalent N-methyl protons show up as a singlet 140 c.p.s. upfield of the HDO peak, while the six-ring



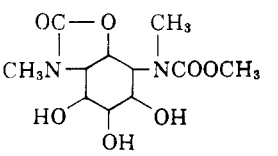
IX



X

protons give two sets of signals centered on 23.6 c.p.s. and 77.0 c.p.s. with relative areas 1:5. The downfield signal is split into a symmetrical triplet (J = 2.6 c.p.s.) and is caused by an equatorial proton split by two equivalent axial *cis* protons on adjacent carbon atoms.[14] The upfield signals are produced by the five axial protons. Data described above and elsewhere[9,10] make IX the more likely structure.

Treatment of actinamine with excess dimethyl carbonate and a trace of base for a prolonged period at reflux temperature produces the bisurethane (XI) and the monoxazolidone (XIII). Compound XI, $C_{12}H_{22}N_2O_8$, shows a single infrared band at 1670 cm.$^{-1}$ which



XIII

(4) Measured at Abbott Laboratories, North Chicago, Ill.

(5) R. L. Peck, C. E. Hoffhine, Jr., E. W. Peel, R. P. Graber, F. W. Holly, R. Mozingo, and K. Folkers, *J. Am. Chem. Soc.*, **68**, 776 (1946).

(6) R. E. Reeves, *J. Am. Chem. Soc.*, **63**, 1476 (1941).

(7) K. H. Meyer and P. Rathgeb, *Helv. Chim. Acta*, **32**, 1102 (1949).

(8) Optical rotatory dispersion measurements were made at Eli Lilly Research Laboratories, Indianapolis 6, Ind., by Dr. M. M. Marsh.

(9) (a) H. Hoeksema, Medicinal Chemistry Symposium, Boulder, Colo., June, 1962; (b) G. Slomp and F. A. MacKellar, *Tetrahedron Letters*, 521 (1962).

(10) L. D. Colebrook and R. H. Gourlay, *Proc. Natl. Acad. Sci., U.S.*, **48**, 1693 (1962).

(11) H. Straube-Rieke, H. A. Lardy, and L. Anderson, *J. Am. Chem. Soc.*, **75**, 694 (1953).

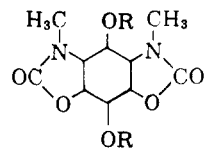
(12) D. H. R. Barton and R. C. Cookson, *Quart. Rev.* (London), **10**, 44 (1956).

(13) Measured at 60 Mc.p.s. on a Varian V-4300B instrument by Dr. L. D. Colebrook.

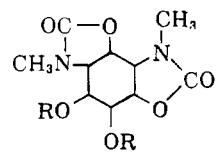
(14) (a) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, p. 85; (b) p. 116.

is in the expected position for an acyclic urethane.[15] The n.m.r. spectrum of XI shows singlet six-proton peaks at 7.13 τ (NCH_3) and 6.38 τ (OCH_3). Acetylation of XI produces a tetraacetate XII, $C_{20}H_{30}N_2O_{12}$, infrared maxima 1750 cm.$^{-1}$ (ester carbonyl), 1700 cm.$^{-1}$ (urethane carbonyl), and 124 cm.$^{-1}$ (acetate ester). Compound XIII, $C_{11}H_{18}N_2O_7$, had infrared maxima at 1760 cm.$^{-1}$ (oxazolidone carbonyl[16]) and 1680 cm.$^{-1}$ (urethane carbonyl[15]). The isolation of this compound with only a single oxazolidone ring is good evidence for a *cis* relationship between the 2-hydroxyl and the 1- or 3-methylamino groups. No trace of product with two oxazolidone rings was obtained under the reaction conditions. Such a compound would be expected if the 4- and 6-hydroxyl groups were also *cis* to the methylamino groups.[17]

The stereochemistry represented by IX is fully confirmed by the isolation of the two isomeric bisoxazolidones XIV and XVI from the reaction of actinamine in aqueous sodium carbonate solution with phosgene.[18] The higher melting isomer XIV, $C_{10}H_{14}N_2O_6 \cdot 2H_2O$, is produced in 3% yield, infrared maxima at 3350 cm.$^{-1}$ and 3100 cm.$^{-1}$ (hydroxyl) and 1738 cm.$^{-1}$ (oxazolidone carbonyl[18]). Its n.m.r. spectrum in pyridine shows only a singlet six proton N-methyl peak at 7.02



XIV. R = H
XV. R = $COCH_3$



XVI. R = H
XVII. R = $COCH_3$

τ. Acetylation of XIV produces diacetate XV, $C_{14}H_{18}N_2O_8$, infrared maxima at 1770 cm.$^{-1}$ (oxazolidone carbonyl[16]), 1750 cm.$^{-1}$ (ester carbonyl), and 1212 cm.$^{-1}$ (acetate ester).

The lower melting isomer XVI, $C_{10}H_{14}N_2O_4$, was isolated in 17% yield as its diacetate XVII, $C_{14}H_{18}N_2O_8$. Acetate XVII had infrared maxima at 1755 cm.$^{-1}$ (ester and oxazolidone carbonyl) and 1226 cm.$^{-1}$ (acetate ester). Sodium methoxide deacetylation of XVII produced XVI, infrared maxima at 3400 cm.$^{-1}$ (hydroxyl), 1725 cm.$^{-1}$ (oxazolidone carbonyl[18]). The n.m.r. spectrum of XVI in deuterium oxide confirms its structure and stereochemistry. The equatorial proton signal 31 c.p.s. downfield from the HDO peak is clearly distinguished from the five axial protons whose signal is centered at 35 c.p.s. upfield of the HDO peak. Furthermore, the N-methyl proton signal at 110 c.p.s. upfield of the HDO peak shows two peaks with a separation of 2.4 c.p.s. This slight splitting due to the non-equivalence of the N-methyl groups would be expected in the unsymmetrical structure, XVI. The absorption of 1.15 moles of periodate per mole of XVI at pH 4.6 and room temperature after eight hours reaction supports the structure assigned to this compound.

The relative yields of XIV and XVI are in keeping with our stereochemical assignments. The formation of XVI involves ring closure with one pair of *cis*-related groups and one pair of *trans*-related groups, whereas the formation of XIV involves ring closure with two pairs of *trans*-related groups. The periodate oxidation of XVI rules out a *trans*-diaxial relationship for the 4- and 5-hydroxyl groups. Our earlier considerations rule out a *cis* relationship for these two groups; the only alternative is represented by IX.

Experimental[19]

Actinospectacin Dihydrochloride.—Commercial actinospectacin dihydrochloride (0.544 g.) was recrystallized from a mixture of water (2 ml.) and acetone (5 ml.) to yield colorless needles, m.p. 200–205°; no change in melting point was observed on repeated recrystallization.

Anal. Calcd. for $C_{14}H_{24}N_2O_7 \cdot 2HCl \cdot 5H_2O$: C, 33.94; H, 7.33; N, 5.66; O, 38.76; Cl, 14.32. Found: C, 34.01; H, 7.55; N, 5.65; O, 38.07; Cl, 14.28.

Actinamine Dihydrochloride.—Actinospectacin dihydrochloride (4.5 g.) was dissolved in 6 *N* hydrochloric acid (80 ml.) and heated for 5 hr. on a steam bath. The dark brown solution was evaporated *in vacuo*, and the resulting solid was redissolved in water and re-evaporated to remove excess acid. The residue was dissolved in the minimum amount of water and treated with a sixfold excess of acetone. After standing for several hours at 5°, colorless needles of actinamine dihydrochloride were filtered off, (2.52 g., 100%), m.p. > 300°. The precipitation procedure was repeated for further purification.

Anal. Calcd. for $C_8H_{18}N_2O_4 \cdot 2HCl$: C, 34.42; H, 7.17; N, 10.03; Cl, 25.45; neut. equiv., 139.6. Found: C, 34.36; H, 7.16; N, 9.81; Cl, 24.77; neut. equiv., 141.5. Titration *vs.* 0.0562 *N* aqueous sodium hydroxide.

Actinamine (I).—Dowex 2 × 4 resin[20] (OH form) (15 g.) was packed into a column 2 cm. in diameter. Actinamine dihydrochloride (3.30 g.) in water (20 ml.) was introduced onto the column and eluted slowly with 300 ml. of water. Evaporation of the eluate *in vacuo* left a sticky residue. Recrystallization from methanol gave white crystals (2.44 g., 93%). Further recrystallizations from aqueous methanol gave pure product (2.33 g., 95% recovery) m.p. 135–136°.

Anal. Calcd. for $C_8H_{18}N_2O_4 \cdot {}^1/_2H_2O$: C, 44.65; H, 8.84; N, 13.02. Found: C, 44.44; H, 8.86; N, 13.10. Neut. equiv. Calcd. for $C_8H_{18}N_2O_4$: 103.1; Found: 102.0. pK_a (1) 9.4; pK_a (2) 7.2.[4] Titration *vs.* 0.0442 *N* aqueous hydrochloric acid.

Actinamine (0.120 g.) was dissolved in cold dilute hydrochloric acid. Addition of a large excess of acetone and cooling to 5° produced colorless needles of actinamine dihydrochloride, m.p. >300° (0.135 g., 86%), infrared spectrum identical with that of authentic material.

Treatment of Actinamine Dihydrochloride with Hydriodic Acid–Red Phosphorus.—Actinamine dihydrochloride (0.104 g.), 47% hydriodic acid (4 ml.), and red phosphorus (0.5 g.) were heated in a sealed tube at 145° for 24 hr.[21] The cooled contents of the tube were filtered through Celite and the filtrate and washings were concentrated *in vacuo*. Iodine was removed by chloroform extraction, and further concentration and cooling to 0° produced colorless needles, m.p. 232–235° dec. This material gave a positive iodide test, was very hygroscopic and rapidly turned brown on exposure to light. Its infrared spectrum was identical with that of actinamine dihydrochloride. These experiments identify the product as actinamine dihydriodide.

Periodate Oxidation of Actinamine.—The uptake of sodium metaperiodate by I was measured at room temperature in a solution buffered to pH 4.6. Standard iodine and sodium arsenite

(15) H. M. Randall, R. G. Fowler, N. Fuson, and J. R. Dangl, "Infrared Determination of Organic Structures," Van Nostrand, New York, N. Y., 1949, pp. 157–158.

(16) H. K. Hall, Jr., and R. Zbinden, *J. Am. Chem. Soc.*, **80**, 6428 (1958), compare the infrared carbonyl frequencies of a number of cyclic and acylic compounds.

(17) E. Dyer and H. Scott, *ibid.*, **79**, 672 (1957), have shown that oxazolidones are the expected products from the treatment of suitable short chain amino alcohols with dialkylcarbonates.

(18) P. R. Steyermark, *J. Org. Chem.*, **27**, 1058 (1962), describes the formation of an oxazolidone from D-glucosamine by this method.

(19) All infrared spectra were determined in Nujol mull or potassium bromide pellets. Melting points are uncorrected, measured in an electrically heated capillary tube. Neutralization equivalents were determined electrometrically using standard saturated calomel and glass electrodes.

(20) Product of J. T. Baker Chemical Co., Phillipsburg, N. J.

(21) J. C. Sheehan, K. Maeda, A. K. Sen, and J. A. Stock, *J. Am. Chem. Soc.*, **84**, 1303 (1962).

TABLE I

PERIODATE OXIDATION OF I

Reaction time, hr.	Moles $NaIO_4$ consumed per mole of I
0.5	4.557
1.5	4.843
3.0	5.241
16.5	5.842
48.0	6.133

solutions were employed[22] to estimate unchanged periodate. Results are summarized in Table I.

No formaldehyde was detected as its dimedone derivative[6] when actinamine (20 mg.) was left with 0.3 *M* sodium periodate (2 ml.) for 6 hr.

Actinamine (0.1781 g.) was allowed to stand with 0.3 *M* sodium periodate (10 ml.) for 2 days. The reaction mixture was then mixed with 25% sodium hydroxide solution (20 ml.) and zinc dust (1 g.) and distilled into dilute hydrochloric acid. Evaporation of the distillate left methylamine hydrochloride (0.0881 g., 1.57 molar ratio) identified by (i) warming with sodium hydroxide solution to produce an ammoniacal gas, (ii) isonitrile test, (iii) chromatography of 5 μl. of a 1% aqueous solution on Whatman no. 1 paper in a 4:1:5 *n*-butyl alcohol–acetic acid–water solvent system, followed by 0.2% ethanolic ninhydrin spray development produced only one spot R_f 0.36, identical in position with that from an authentic sample of methylamine hydrochloride,[23] (iv) conversion to methylphenylthiourea,[24] m.p. 110–111°, no mixture melting point depression with an authentic sample.

Anhydrous I (0.1215 g., 0.59 mmole) reacted with 0.3 *M* sodium periodate (20 ml.) at room temperature for 65 hr. Distillation of this reaction mixture from sodium hydroxide–zinc as above into 0.1153 *N* hydrochloric acid (50.0 ml.) gave a solution which required 42.10 ml. of 0.109 *N* sodium hydroxide on back titration to phenolphthalein end point. Methylamine liberated was 0.03567 g. (1.15 mmoles) or 1.95 moles of methylamine per mole of I.

Hexaacetylactinamine (II).—Actinamine dihydrochloride (0.230 g.) was mixed intimately with powdered freshly fused sodium acetate (0.20 g.) and refluxed with acetic anhydride (15 ml.) for 1 hr. The solvent was removed by evaporation *in vacuo* and the sirupy residue was stirred with water (5 ml.) and left at 0° for several hours. The crude brown crystals which separated (0.35 g., 92%) were recrystallized twice more from aqueous methanol with Norit treatment to yield white cubes of II (0.158 g., 42%), m.p. 205–206°. The same product was obtained from I.

Anal. Calcd. for $C_{20}H_{30}N_2O_{10}$: C, 52.39; H, 6.60; N, 6.11; CH_3CO, 56.20. Found: C, 52.22, 51.99; H, 6.49, 6.70; N, 6.69; CH_3CO, 55.2, 55.8, 57.2.

Hexaacetylactinamine (0.127 g.) was refluxed with 6 *N* hydrochloric acid (10 ml.) for 1 hr. Work-up with hydrochloric acid and acetone as described above gave white needles of actinamine dihydrochloride (0.075 g., 100%), m.p. >300°; infrared spectrum identical to that of an authentic sample.

N,N′-Diacetylactinamine (III). (i) **From II.**—Hexaacetylactinamine (0.90 g.) was dissolved in hot methanol (15 ml.) and treated with 0.1 *N* sodium methoxide (1 ml.).[25] After 24 hr., the first crop of white fluffy crystals (0.272 g.) was removed, and a further crop (0.106 g.) was obtained by concentrating the mother liquors, making the total yield of crude III 66.5%. Two recrystallizations from methanol gave pure III, m.p. 255–256° dec.

Anal. Calcd. for $C_{12}H_{22}N_2O_6$: C, 49.64; H, 7.64; N, 9.65; CH_3CO, 29.65. Found: C, 49.79; H, 7.64; N, 9.84; CH_3CO, 25.3.

(ii) **From I.**—Actinamine (1.04 g.) was dissolved in methanol (35 ml.) and treated with acetic anhydride (5 ml.) at room temperature. After 24 hr. the first crop of crystals was removed (0.451 g.) and a further 0.230 g. was obtained on concentration of the mother liquors to give a total yield of crude III of 50%. This product, after purification, was identical with that obtained from II (mixture melting point and infrared spectrum).

Acetylation of III (0.300 g.) by the sodium acetate method gave, after recrystallization, II (0.300 g., 65%) identical with material obtained directly from I (mixture melting point and infrared spectrum).

Deacetylation of III (0.60 g.) by hot 6 *N* hydrochloric acid (10 ml.) produced actinamine dihydrochloride (0.55 g., 95%) (identified by infrared spectrum).

Periodate Oxidation of III.—The same procedure[22] for estimating periodate was used at 25° and pH 4.6. Formic acid was estimated by slow titration against standard sodium hydroxide using phenolphthalein, bromothymol blue, or thymol blue indicators.[7] The results are summarized in Table II.

TABLE II

PERIODATE OXIDATION OF III

Reaction time, hr.	Moles $NaIO_4$ consumed	Moles HCO_2H produced
0.5	0.347	
1.0	0.609	0.30
4.0	1.506	
8.0	1.934	0.95
20.0	2.262	1.25

N,N′-Dimethyl-O-tetraacetylactinamine (VII).—Actinospectacin (0.60 g.) was heated on the steam bath for 24 hr. with 37% formaldehyde (3.0 ml.), and 90% formic acid (6.0 ml.).[26] Concentrated hydrochloric acid (1.0 ml.) was added to the cooled solution which was then evaporated to dryness *in vacuo*. The residue was dissolved in ethanol and addition of acetone precipitated an amorphous solid (0.57 g.). The crude product was acetylated by refluxing with acetic anhydride (5.0 ml.) and sodium acetate (0.5 g.) for 2 hr. Evaporation to dryness was followed by extraction of the residue with chloroform. The solid product obtained (0.24 g.) was recrystallized twice from methanol to give colorless needles (0.15 g.), m.p. 161–162°, infrared maxima (Nujol) at 1730 $cm.^{-1}$ (ester carbonyl), 1220 $cm.^{-1}$ (acetate ester), and 1048 $cm.^{-1}$ (C—N).

Anal. Calcd. for $C_{18}H_{30}N_2O_8$: C, 53.72; H, 7.50; N, 6.96; CH_3CO, 42.80. Found: C, 53.51; H, 7.36; N, 7.15; CH_3CO, 42.96.

Tribenzoylactinamine (VIII).—Actinamine dihydrochloride (0.54 g.) was dissolved in 10% sodium hydroxide (20 ml.) and shaken with benzoyl chloride (2 ml.) for 15 min. The white sticky precipitate of impure polybenzoate was boiled with methanol to yield a white powder (0.43 g.), m.p. 262–263°. The crude reaction product was refluxed with 0.1 *N* sodium methoxide (15 ml.) for 30 min. Evaporation of the solvent at room temperature left a sticky solid which was recrystallized from hot 80% methanol. Further recrystallizations produced colorless needles of VIII (0.20 g., 55%), m.p. 284–285° dec.

Anal. Calcd. for $C_{29}H_{30}N_2O_7$: C, 67.17; H, 5.83; N, 5.40. Found: C, 67.03; H, 5.92; N, 5.33.

Compound VIII consumed 1.57 moles of periodate over a period of 4 days at room temperature. The oxidation was carried out in methanol using periodic acid. No decay of periodate in a blank solution was observed.

Treatment of I with Dimethyl Carbonate.—Actinamine (0.400 g.) was dissolved in a mixture of methanol (10 ml.) and dimethyl carbonate (2.5 ml.) and refluxed for 7 hr. with triethylamine (3 drops). The solid remaining after evaporation of solvents was neutral to indicator paper and showed infrared maxima (KBr disk) at 1670 $cm.^{-1}$ and 1750 $cm.^{-1}$. Chromatography on Whatman no. 1 paper using 6:4:3 *n*-butyl alcohol–pyridine–water as the solvent system and iodine vapor or 4:1 sodium periodate–potassium permanganate (1% solutions) spray as developing agents gave two spots with R_f 0.35 and 0.62 (*cf.* I R_f 0.21). Four recrystallizations of the reaction product from methanol gave white crystals of XI (100 mg., 17%), m.p. 211–212° dec., R_f 0.62.

Anal. Calcd. for $C_{12}H_{22}N_2O_8$: C, 44.71; H, 6.88; N, 8.69. Found: C, 44.51; H, 6.82; N, 8.34.

Compound XI was also isolated in similar yield when a catalytic amount of sodium methoxide was used in place of the tri-

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(23) Lit. value: R_f 0.37. R. J. Block, R. LeStrange, and G. Zweig, "Paper Chromatography," Academic Press, New York, N. Y., 1952, p. 73.

(24) 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., 1948, p. 227.

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(26) G. F. Holland, R. C. Durant, S. L. Friess, and B. Witkop, *J. Am. Chem. Soc.*, **80**, 6031 (1958).

ethylamine. The mother liquors from the recrystallization of XI were left at 0° for 2 weeks. White crystals of XIII separated (17 mg., 3%), m.p. 222–223°, mixture melting point with XI 206–214° dec.

Anal. Calcd. for $C_{11}H_{18}N_2O_7$: C, 45.51; H, 6.25; N, 9.65; OCH_3, 19.26. Found: C, 45.47; H, 6.14; N, 9.72; OCH_3, 19.40.

Actinamine Bisurethane Tetraacetate (XII).—Actinamine bisurethane (XI) (30 mg.) was acetylated by the sodium acetate method. No product separated on pouring the reaction mixture into water, so the aqueous solution was extracted with chloroform. Evaporation of the dried extract produced an oily solid. This was dissolved in 3:1 petroleum ether–chloroform and chromatographed on Woelm activity III alumina (2 g.). Successive elution with 3:1, 2:1, and 1:1 petroleum ether–chloroform mixtures gave a separation. The material from the 2:1 fractions was recrystallized from methanol to yield pure XII (15 mg., 33%) m.p. 202–203°.

Anal. Calcd. for $C_{20}H_{30}N_2O_{12}$: C, 48.97; H, 6.17; N, 5.71. Found: C, 49.05; H, 6.29; N, 5.65.

Treatment of I with Phosgene.—Compound I (2.403 g., 11.6 mmoles) was dissolved in aqueous sodium carbonate (2.768 g. Na_2CO_3, 22.3 mmoles, in 25 ml. of water).[18] The mixture was cooled externally to 0° in a 100-ml. standard taper joint three-neck flask equipped with mechanical stirrer, Dry Ice–acetone condenser, and soda lime trap. The cold mixture was stirred vigorously and phosgene was introduced slowly over a period of 1 hr., when the reaction mixture had pH ~ 7. After several hours at 0° the products were isolated. (i) The white crystalline deposit of XIV (0.109 g., 0.37 mmole, 3.2%) was filtered off. After recrystallization from aqueous methanol it lost water at 120–140°, darkened at 230°, and melted at 290–291° dec. (0.052 g., 48% recovery).

Anal. Calcd. for $C_{10}H_{14}N_2O_6 \cdot 2H_2O$: C, 40.81; H, 6.17; N, 9.52. Found: C, 40.84; H, 6.10; N, 9.29.

Acetylation of XIV (65 mg.) by the sodium acetate method produced crude brown crystals (73 mg. 97%). Recrystallization from 1:1 methanol–chloroform with Norit treatment produced white crystals of XV (40 mg.), m.p. >315°.

Anal. Calcd. for $C_{14}H_{18}N_2O_8$: C, 49.12; H, 5.30; N, 8.18. Found: C, 48.81; H, 5.28; N, 7.99.

Treatment of XV (25 mg.) with sodium methoxide[25] regenerated XIV (15 mg., 66%) identical in infrared spectrum and giving no mixture melting point depression with authentic XIV. (ii) The filtrate from the phosgene reaction was evaporated to dryness *in vacuo*. The solid residue was acetylated by the sodium acetate method. The resulting solution was concentrated to 10 ml. and water (10 ml.) was then added. Cooling to 0° produced brown crystals (0.668 g., 1.95 mmoles, 17%) which were recrystallized from 1:1 methanol–chloroform with Norit treatment to give small white crystals of XVII (0.423 g.), m.p. 255–256°.

Anal. Calcd. for $C_{14}H_{18}N_2O_8$: C, 49.12; H, 5.30; N, 8.18. Found: C, 49.13; H, 5.59; N, 7.93.

(iii) Compound XVII (0.423 g.) was dissolved in 1:1 methanol–chloroform and treated with 0.1 *N* sodium methoxide (0.5 ml.) overnight.[25] Removal of solvents left a colorless sticky solid which crystallized on trituration with methanol. Recrystallization from hot methanol gave colorless crystals of XVI (0.235 g., 0.91 mmole, 74%), m.p. 226–227°.

Anal. Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.47; N, 10.85. Found: C, 46.56; H, 5.67; N, 10.71.

Acetylation of XVI (28 mg.) by the sodium acetate method produced XVII (19 mg., 55%) after recrystallization, no mixture melting point depression and identical in infrared spectrum with authentic XVII. (iv) Neutralization of the filtrate from the separation of XVII in (ii) with solid sodium bicarbonate caused the precipitation of crude II (1.643 g., 3.58 mmoles, 31%). Two recrystallizations from aqueous methanol gave pure II, identified by mixture melting point and infrared spectrum. Extraction of the mother liquors of the neutral aqueous solution with three 25-ml. portions of chloroform produced a further 1.207 g. (23%) of impure material of low melting point which appeared to be mostly II from inspection of its infrared spectrum.

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Cleavage *vs.* Beckmann Rearrangement in α-Oximino Ketones[1]

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Certain cyclic α-oximino ketones under Beckmann rearrangement conditions yield the expected rearrangement products (imides), but it can be shown that the reaction proceeds first by cleavage followed by ring closure to the imides.

In a previous study[2] we have demonstrated that esters of steroidal α-oximino ketones—*e.g.*, III—undergo facile cleavage with water or alcohols rather than Beckmann rearrangement. Yet the reaction of the corresponding free oxime *anti*-16-oximino-5-androsten-3β-ol-17-one (I) with boiling acetic acid acetic anhydride gave to our surprise mainly imide VIII, a Beckmann rearrangement product. The first step in this reaction is obviously the acylation of I to III and indeed it could be shown that oxime acetate III also was converted to imide VIII under the conditions of the reaction or simply by heating with acetic acid. Based on previous findings[2] we suspected that the oxime acetate III would be cleaved by acetic acid to the mixed anhydride IV which, like the corresponding acid chloride,[2] could then cyclize, possibly *via* V, to imide VIII. When 3β-acetoxy-16-acetoximino-5-androsten-17-one (III) was heated under reflux with acetic acid for a short period of time (one hour) or at a lower temperature (50°) nitrile acid VII was formed in excellent yield but none of the anhydride IV was isolated. Under milder conditions or upon treatment with sodium acetate or propionate in polar solvents, III was recovered unchanged. It was soon established that authentic anhydride IV was much more easily converted by acetic acid to acid VII than was oxime acetate III, and therefore efforts to isolate IV from the reaction of III with acetic acid were doomed to failure.

With the evidence at hand that 3β-acetoxy-5-androstene-16,17-seco-16-nitrile-17-oic acid (VII) may be an intermediate in the conversion of I to VIII, it remained to be established that VII could ring close to imide VIII by heating with acetic acid under reflux. Indeed, heating of oxime acetate III or of nitrile acid VII in acetic acid for twenty-four hours gave an identical product consisting mainly of imide VIII and some 3β-ace-

(1) Presented in part before the National Meeting of the American Association for the Advancement of Science, Denver, Colo., December, 1961.

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