Alanylactinobolone. A Basic Hydrolysis Product of the Antibiotic Actinobolin[1]

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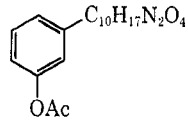
The structure elucidation of *N*-acetylalanylactinobolone, the product derived from the mild basic hydrolysis of *N*-acetylactinobolin, is described. The molecule was shown to possess three secondary hydroxyl groups and a methylene-flanked ketone group ($-CH_2COCH_2-$). Aromatization of the molecule demonstrated the incorporation of the methylene-flanked ketone in a cyclohexane ring and the presence of a side chain array, $CH_3CH(NHCOCH_3)CONHCH(CHOHCH_3)$, β to the carbonyl group. The presence of one of the hydroxyl groups in the side chain demanded placement of the remaining hydroxyl groups at the β' and γ positions of the cyclohexanone ring to give expression **1** for *N*-acetylalanylactinobolone.

The mild basic hydrolysis of the crystalline *N*-acetate of the broad spectrum antibiotic actinobolin[2] leads to the destruction of the parent chromophore, 1 mol of carbon dioxide, and a labile degradation product, *N*-acetylalanylactinobolone, which is the precursor of a second more stable degradation product, *N*-acetylalanylactinobicyclone.[3] The former, *N*-acetylalanylactinobolone, proved to be an important link in the chain of information that led to the elucidation of the structure of the intact antibiotic. Its chemistry, structure, configuration, and preferred conformation comprise the subject of this report.

Elemental analysis and a mass spectral determination establish the moleuclar formula $C_{14}H_{24}N_2O_6$ for *N*-acetylalanylactinobolone (**1**). The presence of three secondary hydroxyl groups is suggested by the nmr spectrum[4a] (dimethyl sulfoxide-d_6) of **1** which displays doublet signals (J = 3.5–4.5 Hz), each equivalent to a single hydrogen, at δ 4.58, 4.98, and 5.04 that rapidly disappear upon addition of deuterium oxide to the nmr probe.[5] Single hydrogen doublets at δ 7.54 (J = 9 Hz) and 8.10 (J = 7 Hz) disappear more slowly and provide evidence for the presence of two secondary amide groups, each bonded to carbon bearing a single hydrogen. Other prominent signals are assigned to *N*-acetate methyl (δ 1.87, s) and to a pair of secondary methyl groups (δ 1.26, d, J = 7 Hz, and δ 1.02, d, J = 6 Hz). The remaining hydrogen resonances fall into one of two discrete sets of overlapping signals; the region from δ 2.5 to 1.9 contains signals for five hydrogens as does the region from δ 4.5 to 3.1. A base-catalyzed deuterium exchange experiment, monitored by nmr, suggested the presence of a $-CH_2COCH_2-$ unit. The absence in the nmr spectrum of a signal characteristic of a methyl ketone militates against the presence of CH_3COCH-. The infrared spectrum of **1**, in addition to the bands associated with hydroxyl and amide groups, displays an unstrained ketone carbonyl band at 1715 cm^{-1}.

Direct esterification as a means of verifying the presence of three secondary hydroxyl groups proved unsuccessful. However, by masking the ketone group as its dimethyl ketal, followed by pyridine-catalyzed acetylation, it was possible to isolate the tri-*O*-acetyldimethyl ketal **2**. Elemental analysis and mass spectral data confirm the molecular formula of **2** as $C_{22}H_{36}N_2O_{10}$. The nmr spectrum of **2** (see Experimental Section) provides support for the required structural features. As expected, the infrared spectrum displays no bands characteristic of ketone or hydroxyl groups.

Acetylation of **1** in acetic anhydride in the presence of perchloric acid leads to an easily isolable product, **3** ($C_{18}H_{24}N_2O_6$).[6] Spectroscopic data (nmr, uv, and ir, see Experimental Section) indicate **3** to be a meta-substituted phenyl acetate. Conversion of the phenyl acetate **3** to the corresponding phenyl methyl ether **5**, followed by basic permanganate oxidation to *meta*-anisic acid confirms the assignment. These data implicate the following partial structure for compound **3**.



Chemical and spectral evidence lead to the arrangement of atoms in the side chain. The vigorous acid hydrolysis of the aromatic compound **3** gives rise to the amino acid alanine. In the nmr spectrum[4a] of **3** (dimethyl sulfoxide-d_6) those signals not associated with the 3-acetoxyphenyl residue indicate the presence of an *N*-acetate and an *O*-acetate methyl group (δ 1.87, 3 H, s, and δ 1.95, 3 H, s, respectively) together with two methyl groups (δ 1.10, 3 H, d, and δ 1.17, 3 H, d), each bonded to carbon bearing a single hydrogen, *i.e.*, CH_3CH-. The presence of two secondary amide groups, each bonded through nitrogen to carbon bearing a single hydrogen, *i.e.*, $-CONHCH-$, is suggested by the two low-field one-hydrogen doublets centered at δ 8.03 and 8.38. The remaining signals appear in the

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(1) (a) Support of this work by the National Institutes of Health through Research Grant AI-04720 is gratefully acknowledged. (b) This paper is based in large part on the Ph.D. Dissertation of D. B. Nelson, Arizona State University. (c) A portion of this work has been published in preliminary form: M. E. Munk, D. B. Nelson, F. J. Antosz, D. L. Herald, Jr., and T. H. Haskell, *J. Amer. Chem. Soc.*, **90**, 1087 (1968).

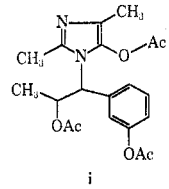
(2) M. E. Munk, C. S. Sodano, R. L. McLean, and T. H. Haskell, *ibid.*, **89**, 4158 (1967).

(3) D. B. Nelson, M. E. Munk, K. B. Gash, and D. L. Herald, Jr., *J. Org. Chem.*, **34**, 3800 (1969).

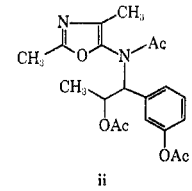
(4) (a) Reported in parts per million (δ) downfield of tetramethylsilane. (b) Reported in parts per million (δ) downfield of 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt.

(5) O. L. Chapman and R. W. King, *J. Amer. Chem. Soc.*, **86**, 1256 (1964).

(6) A chromatographic separation of the mother liquids afforded a second compound. Spectroscopic studies suggest the tentative assignment of structure i or ii; however, the problem has not been pursued.



i



ii

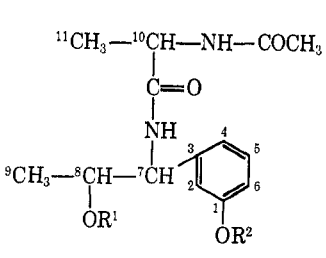
region attributed to hydrogens on carbon bearing oxygen and nitrogen; an unsymmetrical multiplet of two overlapping one-hydrogen signals centered at δ 5.05 and a one-hydrogen quintet at 4.45 (J = 7 Hz).

Exchange of the amide hydrogens for deuterium (addition of deuterium oxide to the sample tube) results in the collapse of the quintet at δ 4.45 to a quartet (J = 7 Hz), an observation consistent with the assignment of this signal to the methine hydrogen of an *N*-acetylalanyl residue, *i.e.*, $CH_3CH(NHCOCH_3)CO$–. A quintet, discernible in the nmr spectrum of compound **1** (dimethyl sulfoxide-d_6) at nearly identical field strength, δ 4.37, behaves similarly upon addition of deuterium oxide. Therefore, it appears unlikely that the signal at δ 4.45 in the spectrum of **3** reflects a benzylic hydrogen, since, in that case, a paramagnetic shift would have been expected for that signal upon aromatization of the ring component of **1**. Additional confirmation of the unit proposed was obtained by double resonance studies[7] which demonstrated coupling between the methine hydrogen quartet at δ 4.45 and the methyl doublet at δ 1.38.

Since a second secondary amide function is required, the unit $CH_3CH(NHCOCH_3)CONH$– is indicated. To complete the side chain array only the point of attachment of this unit and the $-OCOCH_3$ unit to the remaining $CH_3CH<$ and $>CH$ units requires resolution.

Methoxide-catalyzed *O*-deacetylation of compound **3** results in the shift of *one* of the two overlapping nmr signals centered at δ 5.05 to higher field. Addition of deuterium oxide (to exchange hydroxyl and amide hydrogen for deuterium) to the nmr sample tube of the *O*-deacetylated compound **4** in acetone-d_6[4a] leads to the immediate simplification of the diamagnetically shifted signal to a quintet centered at δ 4.07 (J = 6 Hz). The *rapid* resolution of this signal upon addition of deuterium oxide (consistent with *rapid* exchange of hydroxylic hydrogen), its observed multiplicity after deuterium exchange and the diamagnetic shift experienced upon *O*-deacetylation[8] of **3** permits identification of a $CH_3CH(OCOCH_3)$– unit in **3**, *i.e.*, the $-OCOCH_3$ group is assigned to C-8 of the side chain of **3**.

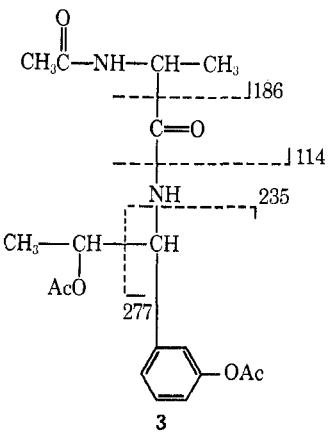
The chemical shift of the *second* of the two signals of the original δ 5.05 multiplet of **3** remains at low field



3, $R^1 = R^2 = COCH_3$
4, $R^1 = R^2 = H$
5, $R^1 = H$; $R^2 = CH_3$

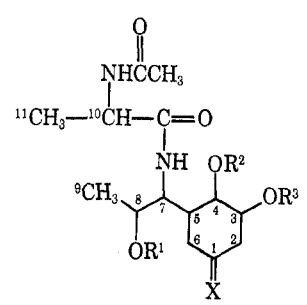
upon *O*-deacetylation and in the presence of deuterium oxide only *slowly* resolves to a doublet centered at δ 4.78 (J = 5 Hz). The slow resolution of this signal (consistent with *slow* exchange of amide hydrogen) allows its assignment to hydrogen on the benzylic carbon (C-7), the carbon atom bearing the *N*-acetylalanylamido group. In this nmr spectrum of **4** the methine proton of the *N*-acetylalanyl residue appears as a quartet centered at δ 4.25 (J = 7 Hz). The implications of these data permit the assignment of structure **3** to the aromatic compound derived directly from acid-catalyzed acetylation of **1**. The well-resolved nmr spectrum of aromatic compound **5**, obtained by treatment of **4** with diazomethane, and the related double resonance experiments (see Experimental Section) provide verification of the assignment.

Further support for the side chain assignment is found in the mass spectrum of **3** which displays peaks at m/e 86 and 114, associated with the *N*-acetylalanyl portion of the side chain, and at m/e 235 and 237 (base peak) resulting from benzylic fragmentation.



3

The structure of the aromatic compound **3** implicates the presence in **1** of these structural features: (1) a cyclohexanone ring, (2) an *N*-(*N*-acetylalanyl)propanolamine side chain, and (3) a β relationship of side chain to the ketone carbonyl, progenitor of the phenolic hydroxyl group. The methylene-flanked ketone (CH_2-$COCH_2$–) and the presence of one hydroxyl group on the side chain require that the remaining two hydroxyl groups occupy the unsubstituted β and γ positions of the cyclohexanone ring. These structural requirements are met by expression **1** for *N*-acetylalanylactinobolone.



1, $R^1 = R^2 = R^3 = H$; $X = O$
2, $R^1 = R^2 = R^3 = COCH_3$; $X = OCH_3, OCH_3$
6, $R^1 = H$; $R^2 = R^3 = (CH_3)_2C<$; $X = O$
7, $R^1 = COCH_3$; $R^2 = R^3 = (CH_3)_2C<$; $X = O$

The formation of an isopropylidene derivative **6**, which can be best characterized as its mono-*O*-acetate **7**, is consistent with the vicinal relationship of two of the hydroxyl groups. In the nmr spectrum[4a] (acetone-d_6) of **7** the low-field signal assigned to H_8 appears as a quartet of doublets ($J_{8,9}$ = 6 Hz, $J_{8,7}$ = 3 Hz)

(7) Field-swept spin decoupling at 60 MHz.
(8) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, Calif., 1964, p 77.

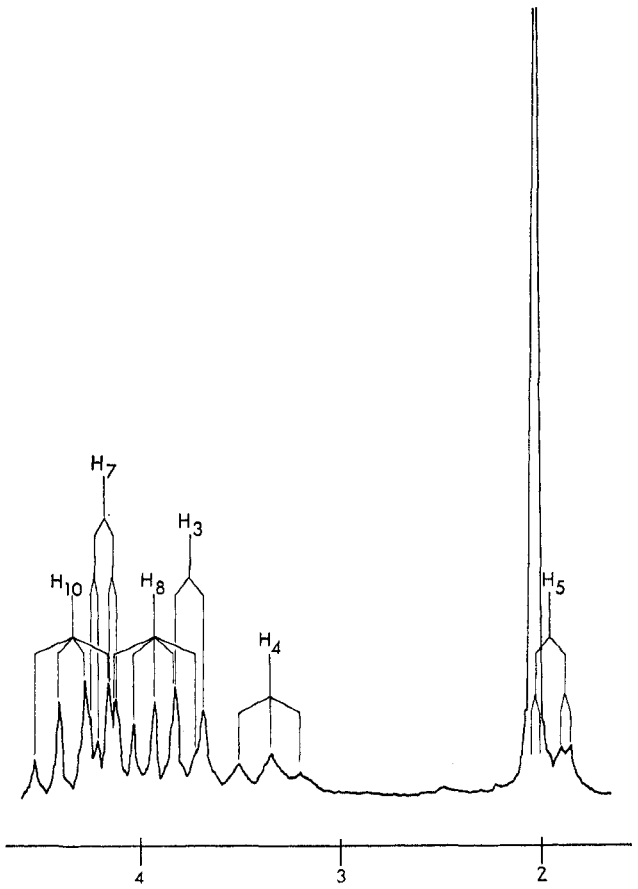
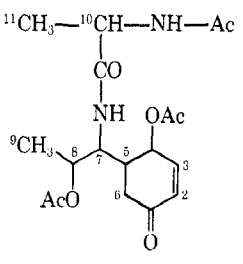


Figure 1.—Nmr spectrum of nonadeuterio *N*-acetylalanylactinobolone.

centered at δ 5.29, an observation consistent with the assignment of the side chain hydroxyl group of **1** to C-8 rather than C-7.

Pyridine-catalyzed acetylation of **1** gives rise to an elimination product which is isolated as its di-*O*-acetate, **8**. The nmr spectrum[4a] (chloroform-*d*) fea-



8

tured well-separated signals amenable to complete analysis (Table I) and spin decoupling studies.

The chemical shifts of the olefinic hydrogens (δ 6.84 and 6.04) and the size of the olefinic coupling constant (J = 10 Hz) are those expected for an α,β-unsaturated cyclohexenone system.[9] Double resonance experiments[7] demonstrate that irradiation of the signal assigned to H_4 results in the collapse of both olefinic hydrogen signals (doublet of doublets) to a simple doublets (J = 10 Hz). Hence, the H_4 signal is coupled equally to the olefinic hydrogens H_2 and H_3. Irradiation of the three-hydrogen signal at δ 2.6 (H_5 and H_6) results in the collapse of the H_4 signal to a triplet (J = 2 Hz). The observed behavior is consistent with the chemical environment of the ring hydrogens in **8**. The nmr data (Table I) also confirm the nature of the side chain array.

(9) See ref 8, p 90.

TABLE I

NMR ASSIGNMENTS, COMPOUND 8[a]

δ	Number of protons	Multiplicity	Assignment
1.25	3	d, $J_{9,8}$ = 6 Hz	$^{9}CH_3$
1.38	3	d, $J_{11,10}$ = 7 Hz	$^{11}CH_3$
1.99	3	s	$-NHCOCH_3$
2.02	3	s	$-^{8}COCOCH_3$
2.19	3	s	$-^{4}COCOCH_3$
2.59	3	m	^{5}CH, $^{6}CH_2$
4.41[b]	1	d of broad d, $J_{7,NH}$ = 9.5, $J_{7,8}$ = 6, $J_{7,5}$ = <1 Hz	^{7}CH
4.63[c]	1	quintet, $J_{10,NH}$ = 8, $J_{10,11}$ = 7 Hz	^{10}CH
5.04	1	quintet, $J_{8,9}$ = 6, $J_{8,7}$ = 6 Hz	^{8}CH
5.40[d]	1	multiplet	^{4}CH
6.04	1	d of d, $J_{2,3}$ = 10, $J_{2,4}$ = 2 Hz	^{2}CH
6.62[e]	1	d, $J_{NH,10}$ = 8 Hz	NH
6.84	1	d of d, $J_{3,2}$ = 10, $J_{3,4}$ = 2 Hz	^{3}CH
7.35[e]	1	d, $J_{NH,7}$ = 9.5 Hz	NH

[a] In $CDCl_3$ *vs.* TMS = 0. [b] Exchange of NH for ND results in a broad doublet, $J_{7,8}$ = 6 Hz, $J_{7,5}$ = <1 Hz. [c] Exchange of NH for ND results in a quartet, $J_{10,7}$ = 7 Hz. [d] When examined in D_2O *vs.* 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt = 0, this signal appears as a doublet of triplets at δ 5.40, $J_{4,5}$ = 9 Hz, $J_{4,3}$ = $J_{4,2}$ = 2 Hz. [e] Signal disappears on addition of D_2O.

The nmr spectrum[4b] of **1** in deuterium oxide containing sodium deuterioxide (to exchange the hydrogens at C-2 and C-6 for deuterium), in which the signals for the five hydrogens on carbon bearing oxygen and nitrogen overlap in the δ 4.7 to 3.0 region (Figure 1), is completely explicable in terms of the structure proposed and, in addition, reveals the preferred conformation of the molecule in solution. After complete deuterium exchange H_3 appears as a doublet at δ 3.77 ($J_{3,4}$ = 8.5 Hz). The H_4 signal is now more clearly visible and appears at δ 3.36 as a triplet ($J_{4,3}$ = $J_{4,5}$ = 8.5 Hz). The magnitude of $J_{3,4}$ and $J_{4,5}$ requires an axial-axial-axial relationship between H_3, H_4, and H_5 of the cyclohexane ring.[10] These observations are consistent with a preference for the chair-like conformation of the cyclohexanone ring of N-acetylalanylactinobolone (**1**) where the substituents at C-3, C-4, and C-5 each occupy the equatorial position.[11] In the α,β-unsaturated ketone **8**, a preference for the half-chair conformation with equatorial-like acetoxyl at C-4 and equatorial-like side chain at C-5 may be inferred from the $J_{4,5}$ value of 9 Hz derived from the H_4 signal (D_2O, see Table I).

A total of three hydrolysis products of the antibiotic actinobolin (**9**) have now been reported: *N*-acetyl-

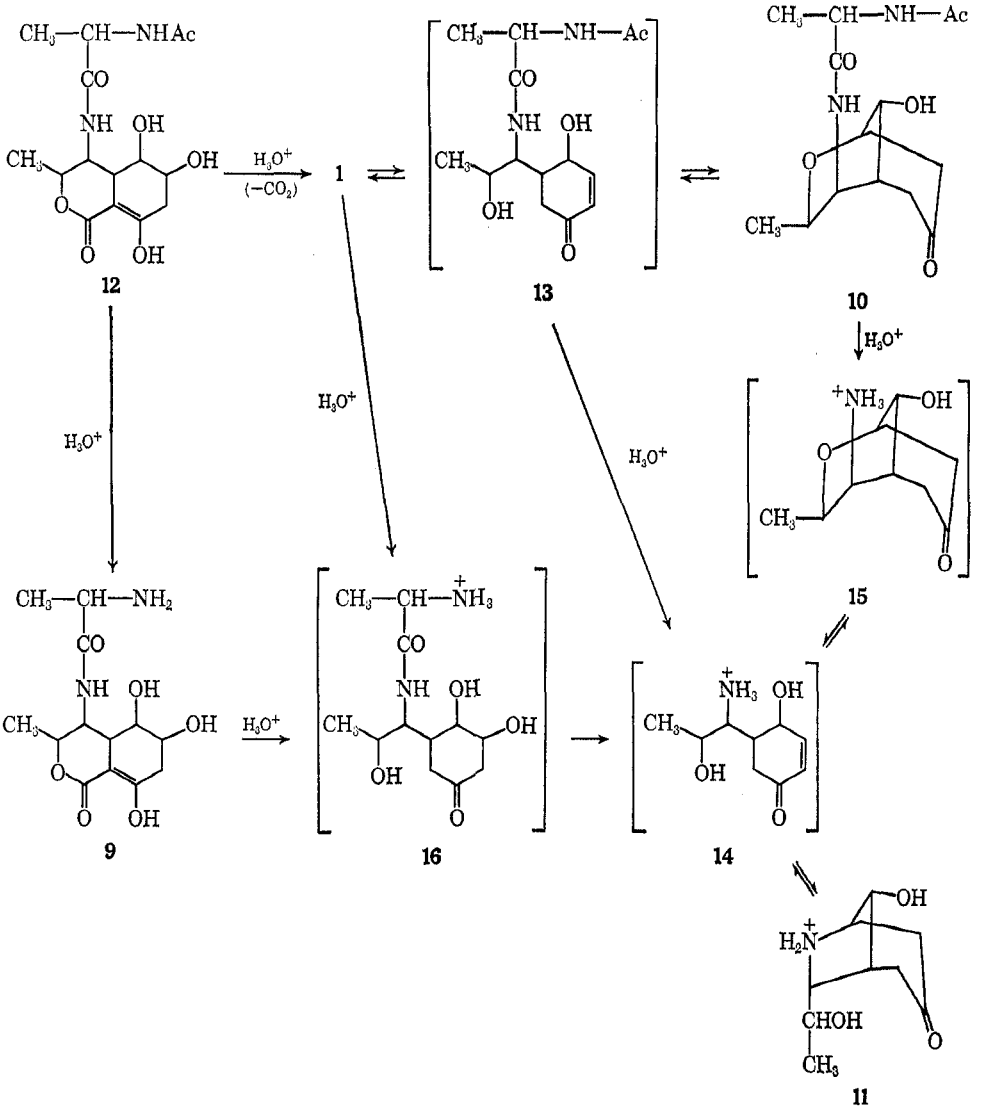
(10) In dimethyl sulfoxide-d_6 solution[4a] the H_4 signal is visible (δ 3.28) as a triplet with a coupling constant of similar magnitude ($J_{4,3}$ = $J_{4,5}$ = 8 Hz).

(11) The recently suggested conformation of *N*-acetylactinobolin,[12] a derivative of the intact antibiotic, is comparable at sites equivalent to C-3, C-4, and C-5. Such an observation together with the mild conditions employed in the cleavage of the lactone ring and subsequent decarboxylation of *N*-acetylactinobolin suggest a retention of configuration in the formation of *N*-acetylalanylactinobolone. Thus, centers C-10, C-8, C-7 C-5, C-4, and C-3 are tentatively assigned as *S*, *R*, *R*, *R*, *R*, and *R*, respectively.

(12) F. J. Antosz, D. B. Nelson, D. L. Herald, Jr., and M. E. Munk, *J. Amer. Chem. Soc.*, **92**, 4933 (1970).

SCHEME I

PATHWAYS OF ACID-INDUCED CLEAVAGE OF ACTINOBOLIN



alanylactinobolone (**1**), *N*-acetylalanylactinobicyclone (**10**),[3] and actinobolamine (**11**).[2] It was of interest to determine the pathways relating these compounds. The original conditions employed in a study of the basic hydrolysis of *N*-acetylactinobolin (**12**), *i.e.*, a 30-min reflux in 1 *N* aqueous ammonia, gave a mixture of **1** and **10** in the ratio of one to two. Under milder conditions, 20 hr at room temperature in 5 *N* aqueous ammonia, both products were again formed but **1** predominated; the observed ratio of **1** to **10** being two to one. Refluxing *N*-acetylactinobolin in 5 *N* aqueous ammonia for 1 hr resulted in the formation of **1** and **10** in the ratio of one to twelve. These observations suggest that the monocyclic system **1** is the direct product of basic hydrolysis of *N*-acetylactinobolin and the precursor of the bicyclic hydrolysis product **10**. The conversion of **1** to **10** during the course of a 30-min reflux period in 1 *N* aqueous ammonia, conditions to which **10** is stable, confirms the base-induced reaction sequence: **12** → **1** → **10**.

A reasonable route for the transformation of **1** to **10** would involve the base-catalyzed β elimination of water to form the α,β-unsaturated ketone **13** followed by base-catalyzed 1,4 addition of the side chain hydroxyl group. The α,β-unsaturated ketone **13** necessary to test the plausibility of this pathway was available as its di-*O*-acetate **8**. Under the mild basic conditions of ester cleavage, 0.1 *N* sodium methoxide in methanol at room temperature, compound **8** was converted to **10**, thus providing support for the elimination–addition sequence **1** → **13** → **10**.

A related α,β-unsaturated ketone, **14**, may serve as the precursor of actinobolamine (**11**), the product derived from the vigorous acid hydrolysis of both actinobolin (**9**)[2] and *N*-acetylalanylactinobicyclone (**10**).[3] Mild acid treatment, 2 *N* sulfuric acid at 60°, results in the conversion of *N*-acetylactinobolin (**12**) to a mixture of *N*-acetylalanylactinobolone (**1**), *N*-acetylalanylactinobicyclone (**10**), and actinobolamine (**11**). Treatment of either **1** or **10** under the same conditions, but for a shorter period of time, leads to a mixture of both compounds. Thus, the interconvertibility of **1** and **10** under acidic conditions, probably *via* the unsaturated ketone **13**, is demonstrated. The possible pathways for acid-induced cleavage of actinobolin and its *N*-acetate are summarized in Scheme I.

No direct or indirect evidence is available to support the intervention of **14**, **15**, or **16**, although, as indicated

above, the α,β-unsaturated ketone **14** appears to be a likely precursor of actinobolamine (**11**), the apparent energy well of the system.

Experimental Section

All melting points are corrected and were taken on a Thomas–Hoover capillary melting point apparatus. Infrared spectra were determined on a Perkin-Elmer Model 237B Infracord and ultraviolet spectra on a Cary Model 14 spectrophotometer. Nuclear magnetic resonance spectra were run in an appropriate solvent on a Varian Associates A-60 spectrometer with tetramethylsilane (TMS) or sodium 3-(trimethylsilyl)-1-propanesulfonate as internal standards and are reported in δ units. Field-sweep decoupling experiments utilized a Varian Associates Model V-6058A spin decoupler. Rotations at the sodium D line were determined on a Rudolf Model 80 polarimeter and optical rotatory dispersion curves were determined with a Jasco Model ORD/UV-5 spectropolarimeter in 10-mm cells. Mass spectra were obtained on an Atlas CH-4B mass spectrometer using a heated direct inlet system, ionizing current of 19 μA, and ionizing energy of 70 eV. Thin layer chromatographic (tlc) plates were prepared with Bio-Sil A(10-30 μ) with 5% binder (purchased from Bio-Rad Laboratories). Mallinckrodt ChromAR sheets (silicic acid) were used for preparative tlc. Solvent systems for tlc and visualization methods are listed where used. Column chromatography separations were performed with Bio-Sil A, 100–200 mesh, silicic acid (purchased from Bio-Rad Laboratories). Microanalyses were performed by Midwest Microlab, Inc., Indianapolis, Ind.

Basic Hydrolysis of Actinobolin. Determination of Carbon Dioxide.—A three-necked flask was fitted with a dropping funnel, a West condenser, and a gas inlet. Nitrogen gas was passed through an Ascarite-filled tube into the reaction flask, exited through the condenser, passed through a drying clyinder containing concentrated sulfuric acid and into a removable tube containing Ascarite and magnesium perchlorate (protected from the atmosphere by a two-stage mineral oil-filled gas exit). Carbon dioxide liberated in the reaction flask was swept into and adsorbed in the Ascarite magnesium perchlorate tube and determined by weight difference. The apparatus was shown to provide a reasonably accurate (5%) estimate of carbon dioxide resulting from the acidification of carbonate salt solutions and carbon dioxide liberated in the acid hydrolysis of actinobolin[2] (1 mol of carbon dixode *per* mol of actinobolin). An example of the procedure used in determining carbon dioxide liberated in the basic hydrolysis of actinobolin is described below.

After addition to the reaction flask of 368.4 mg (1.00 mmol) of actinobolin sulfate the flask was flushed under slow nitrogen flow. The Ascarite magnesium perchlorate tube was removed, weighed, and reconnected as 25 ml of 1 *N* ammonium hydroxide was added to the reaction flask. The flask was heated to reflux and maintained at reflux for 30 min under a slow nitrogen flow. The heating mantle was removed, the flask allowed to cool, and a 1 *N* hydrochloric acid solution containing phenolphthalein was added *via* the dropping funnel until the reaction solution was slightly acidic. The reaction flask was allowed to remain at room temperature for 4 hr under a slow nitrogen flow. The Ascarite magnesium perchlorate tube was removed and weighed. The weight difference indicated 42.2 mg of carbon dioxide (0.96 mmol).

Preparation of *N*-Acetylalanylactinobolone (1).—A solution of 1.475 g (4.32 mmol) of *N*-acetylactinobolin in 45 ml of 5 *N* ammonium hydroxide was stirred for 20 hr at room temperature. The solution was passed over a column containing 15 ml of Bio-Rad AG 21-K anion-exchange resin (hydroxide form), and the water eluent was freeze-dried to yield a residue of 1.128 g. The freeze-dried solid was adsorbed onto silicic acid, dry loaded into a column containing 90 g of silicic acid and eluted with ethyl acetate containing increasing amounts of ethyl alcohol. Elution with ethyl acetate–ethyl alcohol (25/4, v/v) gave first a homogeneous product band followed by a mixed component zone and then a second homogeneous product band. Crystallization of the first band from ethyl acetate gave 298 mg of *N*-acetylalanylactinobicyclone. The second homogenous band on crystallization from acetone gave 627 mg (46%) of *N*-acetylalanylactinobolone (1), mp 161.5–162.5° (resolidifies and melts at 178–180°). Vacuum drying gave an analytical sample: mp 179–180°; $[\alpha]^{25}D$ −57.1° (*c* 3.1, H_2O); ν_{max}^{KBr} 3550–3250 (broad, OH and amide NH), 3070 (amide NH), 1715 (unstrained ketone C=O), 1665 and 1640 (amide C=O), 1540 cm^{-1} (amide II); ORD (*c* 3.7, MeOH) negative plain curve; nmr (DMSO-d_6) (see discussion) (D_2O) δ 4.7–3.0 (5 H, m, hydrogen on carbon bearing heteroatoms), 3.0 to 1.7 (5 H, m, methine H and CH_2COCH_2), 2.05 (3 H, s, $NCOCH_3$), 1.43 (3 H, d, J = 7 Hz, $CHCH_3$), and 1.19 (3 H, d, J = 6 Hz, $CHCH_3$);[13] mass spectrum *m/e* (rel intensity) 316 (<1), 298 (6), 272 (27), 186 (21), 168 (44), 158 (69), 141 (61), 140 (56), 131 (53), 123 (41), 114 (100), 87 (93), 86 (87).

Anal. Calcd for $C_{14}H_{24}N_2O_6$: C, 53.15; H, 7.65; N, 8.86; O, 30.35; mol wt, 316. Found: C, 53.09; H, 7.73; N, 8.88; O, 30.46; mol wt, 316 (mass spectrum).

N-Acetylalanylactinobolone Dimethyl Ketal Tri-*O*-acetate (2).—The procedure of Lorette and Howard[14] was used. A solution of 121 mg (0.38 mmol) of **1**, 12.3 g of dimethoxypropane ($n^{25}D$ 1.2748), 5 mg of *p*-toluenesulfonic acid monohydrate, and 10 ml of methanol was refluxed for 45 min, then set for distillation. After 15 ml of distillate had been collected at ~60°, 10 ml of methanol was added to the solution and an additional 10 ml of distillate taken. On cooling, 1 ml of methanol-washed Bio-Rad AG 21-K anion-exchange resin (hydroxide form) was added to the pot liquid. The mixture was stirred and filtered. The filtrate was taken to dryness under reduced pressure to give 137 mg of a clear film whose ir (film from methanol) displayed no ketone carbonyl stretch.

A solution composed of the filtrate film, 1.5 ml of acetic anhydride and 1.5 ml of pyridine was allowed to sit at room temperature for 12 hr. Removal of solvent under reduced pressure gave an oil which crystallized from ethyl acetate-methylcyclohexane to give 123 mg (64%) of **2**, mp 173–175°. Recrystallization gave analytically pure material: mp 176–178°; $[\alpha]^{26}D$ +6.8° (*c* 4.3, MeOH); nmr (DMSO-d_6) three of the five hydrogen signals in the δ 4.5–3.1 region of the spectrum of **1** experience a paramagnetic shift[8] and appear as an overlapping set of signals in the δ 5.1–4.5 region, other pertinent signals appear at δ 1.88 (3 H, s, $-OCOCH_3$), 1.91 (3 H, s, $-OCOCH_3$), 1.93 (3 H, s, $-OCOCH_3$), 3.10 (3 H, s, OCH_3), and 3.15 (3 H, s, OCH_3); mass spectrum *m/e* (rel intensity) 457 (<1), 428 (<1), 397 (<1), 369 (8), 309 (83), 190 (51), 164 (61), and 136 (100).

Anal. Calcd for $C_{22}H_{36}N_2O_{10}$: C, 54.08; H, 7.43; N, 5.74; O, 32.75; mol wt, 488. Found: C, 54.08; H, 7.56; N, 5.64; O, 32.77; mol wt, 488 (mass spectrum *m/e* 457, 488 $-OCH_3$).

Acetylation of 1. Preparation of the Aromatic *O*-Acetate 3.—To a three-necked flask equipped with a gas inlet, a pressure-equalizing dropping funnel containing 70% perchloric acid, and a gas outlet was added 250 mg (0.79 mmol) of **1** and 4 ml of acetic anhydride. The slurry was stirred magnetically at 0° for 30 min under nitrogen flow. A drop of perchloric acid was added and followed 5 min later with a second drop. The solution was allowed to come to room temperature over a period of 20 min and poured onto ice and the water layer extracted three times with 25-ml portions of dichloromethane. The combined dichloromethane extracts were dried over anhydrous magnesium sulfate and filtered. Volatile solvent was removed from the filtrate under reduced pressure. Further solvent removal under high vacuum left a crystalline mass which was triturated with cold ethyl acetate and filtered to give 33 mg of **3**, mp 141–143°. The filtrate was loaded into a column containing 20 g of silicic acid. Elution with ethyl acetate gave first a difficult-to-crystallize oil[6] followed by cuts containing 85 mg of readily crystallizable **3**, mp 141–143°, to bring the crude yield of **3** to 118 mg (41%). Recrystallization from ethyl acetate gave analytically pure material: mp 146–147°; $[\alpha]^{25}D$ −79° (*c* 4.1, MeOH); λ_{max}^{EtOH} 262, 269 mμ (ϵ 300, 246);[15] ν_{max}^{KBr} 1770 (aromatic acetate

(13) Addition of sodium deuteroxide to a chilled solution of **1** in deuterium oxide (Figure 1) results in the immediate loss of signals equivalent to four hydrogens in the δ 3.0–1.7 region (leaving a signal at δ 1.98) and the simplification of the δ 4.7–3.0 region. The signal assignment for H_3 and H_4 discussed in the text can then be made, as can the assignment of H_{10} at δ 4.35 (1 H, q, $J_{10,11}$ = 7 Hz), H_7 at δ 4.20 (1 H, d of d, $J_{7,8}$ = 6 Hz, $J_{7,5}$ = 2.5 Hz), H_8 at δ 3.94 (1 H, quintet, $J_{8,9} = J_{8,7}$ = 6 Hz), and H_5 at δ 1.98 (1 H, d of d, $J_{5,4}$ = 8.5 Hz, $J_{5,7}$ = 2.5 Hz). Assignment of side-chain methine signals is aided by spin decoupling studies[7] which fortuitously allow the decoupling of both H_{10} from H_{11}, and H_8 from H_9 at the same chemical shift difference. The H_7 pattern is then clearly visible as a doublet of doublets while H_{10} appears as a singlet and H_8 is a doublet ($J_{8,7}$ = 6 Hz).

(14) N. B. Lorette and W. L. Howard, *J. Org. Chem.*, **25**, 521 (1960).

(15) Found for *m*-cresyl acetate: λ_{max}^{EtOH} 262, 269 mμ (ϵ 324, 296).

C=O),[16] 1735 (aliphatic acetate C—O), 1630 (amide (C=O), 1540 (amide II), 1210 cm^{-1} (C—O)[16]; nmr (DMSO-d) δ 8.38 (1 H, d, J = 9 Hz, NH), 8.03 (1 H, d, J = 7 Hz, NH), 7.2 (4 H, aromatic),[17] 5.05 (2 H, m, H-7, H-8), 4.45 (1 H, quintet, J = 7 Hz, H-10), 2.28 (3 H, s, ArOCOCH$_3$),[18] 1.95 (3 H, s, OCOCH$_3$), 1.86 (3 H, s, NHCOCH$_3$), 1.17 (3 H, d, J = 7 Hz, H-11), 1.10 (3 H, d, J = 6 Hz, H-9); mass spectrum m/e (rel intensity) 364 (<1), 277 (100), 235 (1), 164 (74), 114 (5), 87 (7), 86 (5).

Anal. Calcd for $C_{18}H_{24}N_2O_6$: C, 59.33; H, 6.64; N, 7.69; mol wt, 364. Found: C, 59.58; H, 6.69; N, 7.59; mol wt (mass spectrum), 364.

Deacetylation of 3. Preparation of the Phenol 4.—A solution of 348 mg (0.96 mmol) of **3** in 15 ml of 0.1 N sodium methoxide in methanol was stirred at room temperature for 35 min. The reaction solution was passed through a column containing 20 ml of methanol-washed Amberlite 120 cation-exchange resin (proton form). The eluent and column washing were combined and taken to dryness under reduced pressure to give 198 mg (74%) of **4**: homogeneous to tlc (acetone, H_2SO_4 char); λ_{max}^{EtOH} 275, 280 mμ (ϵ 1870, 1700); $\nu_{max}^{EtOH,\ -OH}$ 294 mμ (ϵ 2950); nmr (acetone-d_6) δ 1.11 (3 H, d, J = 6 Hz, H-9), 1.30 (3 H, d, J = 7 Hz, H-11), 1.96 (3 H, s, NCOCH$_3$), 4.12 (2 H, H-^{8}C-OH), 4.58 (1 H, quintet, J = 7 Hz, H-10), 4.81 (1 H, d of d, J = 9 and 5 Hz, H-7), 7.4 to 6.5 (4 H, aromatic,[17] 7.59 (1 H, d, J = 8 Hz, NH), 7.82 d, J = 9 Hz, NH), and 8.47 (1 H, s, phenol OH). Addition of D_2O causes signals at δ 8.47, 7.82, and 7.59 to disappear while these signals are altered: δ 4.07 (1 H, quintet, J = 6 Hz, H-8), 4.52 (1 H, q, J = 7 Hz, H-10), and 4.78 (1 H, d, J = 5 Hz, H-7).

Methylation of 4. Preparation of the Methyl Ether 5.—To a solution of 138 mg (0.49 mmol) of **4** and **3** ml of methanol in a 25-ml round-bottomed flask fitted with a Dry Ice condenser was added ~300 mg of diazomethane in 15 ml of ether and the resulting solution was stirred at room temperature for 8 hr. Removal of solvent under reduced pressure gave 152 mg of a slightly yellow solid. Crystallization from ethyl acetate gave 120 mg (83%) of **5** as analytically pure material: mp 134–135°; $[\alpha]^{26}D$ −120° (c 4.9, MeOH); λ_{max}^{EtOH} 212 mμ (ϵ 8900), 272 (2000), 280 (1900); nmr (D_2O) δ 7.6 to 6.8 (4 H, aromatic), 4.85 (1 H, d, J = 5.5 Hz, H-7), 4.45 (1 H, q, J = 7 Hz, H-10), 4.20 (1 H, quintet, J = 6 Hz, H-8), 3.86 (3 H, s, OCH$_3$), 2.12 (3 H, s, NCOCH$_3$), 1.38 (3 H, d, J = 7 Hz, H-11), 1.19 (3 H, d, J = 6.5 Hz, H-9); spin decoupling studies[7] (signal irradiated, signal observed, multiplicity change) H-8, H-9, d → s; H-8, H-7, d → s; H-7, H-8, quintet → q (J = 6 Hz), H-9, H-8, quintet → d (J = 5 Hz); mass spectrum m/e (rel intensity) M + 1 = 295 (4), 276 (8), 251 (24), 250 (100), 249 (27), 232 (16), 191 (20), 163 (24), 136 (35), 114 (22), 91 (13), 87 (17), 86 (18).

Anal. Calcd for $C_{15}H_{22}N_2O_4$: C, 61.20; H, 7.54; mol wt, 294. Found: C, 60.97; H, 7.54; mol wt, 294 (mass spectrum, M + 1 = 295).

Oxidation of 5 to *meta*-Anisic Acid.—A solution of 105 mg (0.36 mmol) of **5**, 330 mg of potassium permanganate, and 210 mg of sodium hydroxide in 2 ml of H_2O was heated at steam bath temperature for 1 hr, an additional 2 ml of H_2O added, and heating continued for 2 hr. The reaction solution was cooled and filtered. The filtrate and the water washes were combined and acidified with concentrated sulfuric acid. Sodium bisulfite was added until color decreased to a constant level, the solution cooled and extracted three times with 25-ml portions of ether. The ether extracts were combined, dried over anhydrous magnesium sulfate and filtered. The filtrate was taken to dryness under reduced pressure to give 75 mg of a tan residue. A tlc examination (EtOAc, bromocresol green visible) revealed a mixture of acidic components. Preparative tlc on an 1 ft × 8 ft Chrom-AR-1000 sheet (developed with EtOAc; visible *via* uv) revealed a major band with R_f comparable with *meta*-anisic acid. The major band was cut out and eluted with ethyl acetate–ethyl alcohol (3:1, v/v). Removal of solvent under reduced pressure gave 34 mg of a slightly colored crystalline mass. Purification by vacuum sublimation gave 20 mg of *meta*-anisic acid, mp 106–107° (lit.[19] mp 109–110°). The ir spectrum could be superimposed upon that of authentic *meta*-anisic acid, and the nmr ($CDCl_3$) was identical with an nmr of *meta*-anisic acid.

Preparation of the Isopropylidene Derivative of N-Acetylactinobolin.—In a 500-ml round-bottom flask fitted with a Soxhlet condenser containing Linde 3A Molecular Sieves in the thimble was placed 1.193 g (3.48 mmol) of N-acetylactinobolin, 250 ml of acetone, and 60 mg of p-toluenesulfonic acid monohydrate. The solution was refluxed for 22 hr, cooled, and passed through a column containing 20 ml of Amberlite IR-45 weakly basic anion-exchange resin. The eluent was taken to dryness under high vacuum. The resultant solid was triturated with cold acetone and filtered to give 657 mg of N-acetylactinobolin, identified by tlc (ethyl acetate–ethyl alcohol, 2:1, v/v). The filtrate was loaded into a column containing 20 g of silicic acid and eluted with acetone. The first 125 ml of acetone contained 557 mg (1.46 mmol) of the isopropylidene derivative of N-acetylactinobolin. Further elution gave an additional 13 mg of N-acetylactinobolin to bring the yield of recovered N-acetylactinobolin to 670 mg (1.96 mmol). The crude yield of product based on starting material utilized was 95%. Recrystallization from acetone followed by vacuum drying at 78° gave an analytical sample: mp 238–240° dec; $[\alpha]^{26}D$ + 26.3 (c 3.6, MeOH); λ_{max}^{EtOH} 262 mμ (ϵ 9000); $\lambda_{max}^{EtOH,\ OH^-}$ 287 mμ (ϵ 17,400); ν_{max}^{KBr} 3340, 3300–3250 and 3055 (amide NH), 1687, 1660–1630 (C=O), 1625 (amide C=O), 1525 (amide II), 1390, 1380 cm^{-1} (gem-methyl).

Anal. Calcd for $C_{18}H_{26}N_2O_7$: C, 56.53; H, 6.85; O, 29.29; mol wt, 382. Found: C, 56.71, H, 6.99; O, 29.13; mol wt, 382 (mass spectrum).

Preparation of 6. A. Hydrolysis of Isopropylidene N-Acetylactinobolin.—A solution of 380 mg (0.95 mmol) of isopropylidene N-acetylactinobolin in 25 ml of 1 N ammonium hydroxide was refluxed for 35 min, cooled, and freeze-dried. The freeze-dried residue was diluted with water and passed over a column of 5 ml of Bio-Rad AG 21-K anion-exchange resin (hydroxide form). The water eluents were freeze-dried to give 262 mg. Crystallization from acetone gave 131 mg; chromatography of the mother liquor on silicic acid using ethyl acetate–ethyl alcohol (25:2, v/v) as eluent gave an additional 50 mg of **6** for a total yield of 181 mg (51%): mp 160–161°; nmr (DMSO-d_6) δ 4.6 (1 H, d, ^{8}C-OH, diappears on addition of D_2O), 1.37 (6 H, gem-methyl). It should be noted that the presence of **10** could not be detected in the hydrolysis mixture.

B. From 1.—In a 25-ml round-bottom flask fitted with a micro Soxhlet containing Linde 3A molecular Sieves in the thimble was placed 23 mg (0.07 mmol) of **1**, 15 ml of acetone, and 8 mg of p-toluenesulfonic acid monohydroate. The solution was refluxed for 26 hr, cooled, and passed over a column containing 5 ml of Amberlite IR-45 weakly basic anion-exchange resin. The eluent was taken to dryness under vacuum. A tlc examination (ethyl acetate–ethyl alcohol, 2:1, v/v, sulfuric acid char) revealed the presence of **1**, **6**, and N-acetylalanylactinobicyclone (**10**). The formation of **6** (in ~35% yield) and **10** was confirmed by isolation *via* silicic acid column chromatography and comparison of the ir spectrum of each product with an ir spectrum of authenic **6** and **10**.[3]

Preparation of Isopropylidene N-Acetylalanylactinobolone O-Acetate (7).—A solution of 222 mg (0.63 mmol) of **6** in 4 ml of acetic anhydride and 4 ml of pyridine was stirred at 5° for 12 hr. solvent was removed under reduced pressure to give a white solid which crystallized from acetone–methylcyclohexane to give 183 mg (80%) of **7**, mp 145–147°. Recrystallization and extensive drying gave a sample for analysis: mp 147–148°; $[\alpha]^{25}D$ −25.0 (c 4.1, MeOH); ν_{max}^{KBr} 1730 (acetate C=O), 1715 (ketone C=O), 1375 cm^{-1} (gem-dimethyl); nmr (acetone-d_6) δ 5.29 (1 H, q of d, J = 6 and 3 Hz, H-8), 1.37 (3 H, s, gem CH$_3$), 1.42 (3 H, s, gem CH$_3$), 1.98 (3 H, s, OCOCH$_3$), and 1.95 (3 H, s, -NHCOCH$_3$); mass spectrum m/e (rel intensity) peaks characteristic of an isopropylidene group[20] at 383 (4) M − CH$_3$ 340 (2) M − CH$_3$COCH$_3$, and 323 (1) M − (CH$_3$ + CH$_3$CO$_2$H).

Anal. Calcd for $C_{19}H_{30}N_2O_7$: C, 57.27; H, 7.59; O, 28.12; mol wt, 398. Found: C, 56.74; H, 7.67; O, 27.96; mol wt, 398 (mass spectrum, M + 1 = 399).

Acetylation of 1 under Basic Conditions. Preparation of the α,β-Unsaturated Ketone 8.—A solution of 202 mg (0.64 mmol) of

(16) Ascribed to an aromatic acetoxy unit: see K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, San Francisco, Calif., 1962, p 44.

(17) The nature of the aromatic substitution pattern is most clear in the nmr of **4** (acetone-d_6) which displays a four hydrogen aromatic region very similar in chemical shift and pattern to that reported for *m*-cresol. "Varian Spectra Catalog," Varian Associates, Palo Alto, Calif., 1962, number 160.

(18) A chemical shift about 0.3 ppm downfield of the methyl group of an aliphatic acetate. See ref 8, p 98.

(19) E. H. Rodd, "Chemistry of Carbon Compounds," Vol. III, Part B, Elsevier, Amsterdam, 1956, p 768.

(20) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectroscopy," Vol. II, Holden-Day, San Francisco, Calif., 1964, p 228.

1 in 2 ml of acetic anhydride and 2 ml of pyridine was heated at steam bath temperature for 3.5 hr under a nitrogen blanket. Solvent was removed under reduced pressure and the resulting residue was loaded into a column containing 25 g of silicic acid. Elution with benzene–ethyl alcohol (95:5, v/v) gave a homogeneous material which was crystallized from benzene to give 137 mg of **8** holding benzene as solvent of crystallization. Drying to constant melting point gave analytically pure material: mp 131–132°; $[\alpha]^{26}D$ +51° (*c* 5.2, MeOH); λ_{max}^{EtOH} 211 (ε 9500); ν_{max}^{KBr} 1750 and 1730 (acetate C=O), 1685 cm^{-1} (ketone C=O); mass spectrum *m/e* (rel intensity) M + 1 = 383 (<1), 322 (1), 295 (16), 235 (35), 182 (85), 150 (35), 123 (45), 122 (100), 116 (71), 114 (72), 87 (72), 86 (69); nmr, see discussion.

Anal. Calcd for $C_{18}H_{26}N_2O_7$: C, 56.53; H, 6.85; O, 29.29; mol wt, 382. Found: C, 56.35; H, 6.95; O, 29.34; mol wt, 382 (mass spectrum, M + 1 = 383).

Basic Hydrolysis of Actinobolin.—A solution of 1.602 g (4.42 mmol) of the sulfate salt of actinobolin in 75 ml of 1 *N* ammonium hydroxide was refluxed for 35 min, cooled, and passed through a column containing 40 ml of Bio-Rad AG 21-K anion-exchange resin (hydroxide form). The water eluent was freeze-dried and the resulting solid was taken up in 25 ml of ethyl alcohol contining 1.5 ml of acetic anhydride. The solution was stirred at room temperature for 12 hr. Solvent was removed *via* high vacuum and the resulting clear glass was adsorbed onto silicic acid, dry loaded into a column containing 50 g of silicic acid and eluted with ethyl acetate containing increasing amounts of ethyl alcohol. Elution with ethyl acetate–ethyl alcohol (25:3, v/v) gave 616 mg of crystalline solid whose physical and spectral properties were identical with those of *N*-acetylalanylactinobicyclone (**10**), isolated from the basic hydrolysis of *N*-acetylactinobolin. Further elution with ethyl acetate–ethyl alcohol (25:4, v/v) gave cuts which contained a second less mobile product. Rechromatography of the mixed product cuts resulted in the isolation of an additional 17 mg of **10** and 112 mg of a second product whose physical and spectral properties were identical with those of *N*-acetylalanylactinobolone (**1**) isolated from the basic hydrolysis of *N*-acetylactinobolin.

Basic Hydrolysis of 1.—A solution of 5 mg of **1** in 5 ml of 1 *N* ammonium hydroxide was refluxed for 35 min, cooled, and freeze-dried. A tlc examination (ethyl acetate–ethyl alcohol, 2:1, v/v, sulfuric acid char) revealed the presence of a single compound, *N*-acetylalanylactinobicyclone (**10**). In a parallel experiment **10** was shown to be stable to the reaction conditions.

Methanolysis of 8.—A solution of 67 mg (0.18 mmol) of **8** in 4 ml of 0.1 *N* sodium methoxide in methanol was stirred at room temperature for 30 min and passed through a column containing 5 ml of methanol-washed Amberlite 1R-120 cation-exchange resin (proton form). The eluent was taken to dryness under reduced pressure. A tlc examination (Merck HF 254,[21] ethyl acetate–ethyl alcohol, 2:1 v/v, sulfuric acid char) revealed the absence of **8** and the presence of *N*-acetylalanylactinobicyclone (**10**) as the major product. Preparative tlc (ethyl acetate–ethyl alcohol, 2:1, v/v) of the methanolysis products gave 31 mg (0.10 mmol) of **10** identified by melting point and ir.

Vigorous Acid Hydrolysis of 1.[22]—A solution of mg of **1** in 2 ml of 4 *N* sulfuric acid was refluxed for 15 hr, cooled, and passed through a column containing 15 ml of Bio-Rad AG 21-K anion-exchange resin (hydroxide form). The column was eluted with water until the eluents were neutral and then with a 10% acetic acid solution. The residue left after freeze-drying of the water eluent was *N*-acetylated in ethyl alcohol–acetic anhydride. After removal of solvent *via* high vacuum a tlc examination (ethyl acetate–ethyl alcohol, 2:1, v/v, sulfuric acid char) demonstrated the product to be the *N*-acetyl derivative of actinobolamine (**11**).[2] A tlc examination (iso propyl alcohol, pyridine, acetic acid, water, 8:8:1:1, v/v/v/v and methyl ethyl ketone, propionic acid, water, 75:25:30, v/v/v, ninhydrin visible) of the residue left after freeze-drying of the acetic acid eluent demonstrated the product to be alanine.

Vigorous Acid Hydrolysis of 3.[22]—Hydrolysis of 2 mg of **3** using the conditions described for **1**, followed by a parallel work-up led to an amphoteric product which was shown to be alanine by a tlc examination.

Mild Acid Hydrolysis of *N*-Acetylalanylactinobolone (1).—A solution of 12 mg of **1** in 5 ml of 2 *N* sulfuric acid was heated at 60°. After 0.5 hr (1 ml), 1.5 hr (2 ml), and 2.5 hr (2 ml) of heating, a sample was removed, diluted with water, stirred with 10 ml of Bio-Rad AG 21-K anion-exchange resin (hydroxide form), and filtered. The filtrates were freeze-dried and the resulting residues examined *via* tlc (ethyl acetate–ethyl alcohol, 2:1, v/v, sulfuric acid char). All samples revealed the absence of low R_f material (*i.e.*, **11**), the presence of **1**, and the presence of **10** (as the major product at 1.5 and 2.5 hr).

Mild Acid Hydrolysis of *N*-Acetylalanylactinobicyclone (10).—A solution of 11 mg of **10** in 5 ml of 2 *N* sulfuric acid was heated at 60°. Samples were removed, worked up, and examined *via* tlc as in the mild acid hydrolysis of **1**. All samples revealed the absence of low R_f material, the presence of **10** as the major product, and the presence of a lesser amount of **1** at 1.5 and 2.5 hr.

Mild Acid Hydrolysis of *N*-acetylactinobolin (12).[23]—A solution of 22 mg of **12** in 2 ml of 2 *N* sulfuric acid was heated at 60° for 27 hr, cooled, and passed through a column containing 15 ml of Bio-Rad AG 21-K anion-exchange resin (hydroxide form). The water eluent (75 ml) was freeze-dried. The resulting residue was dissolved in water and passed through a column containing 10 ml of Bio-Rad AG 50W-X8 cation-exchange resin (proton form). The column was eluted with water (70 ml) and 5% aqueous ammonia (100 ml). The solid left after freeze-drying of the basic eluent was *N*-acetylated in 1 ml of ethyl alcohol containing 0.5 ml of acetic anhydride. A tlc examination (ethyl acetate–ethyl alcohol, 2:1 and 3:1, v/v, sulfuric acid char) indicated the neutral fraction (freeze-dried water eluent) contained both **1** and **10** while the *N*-acetylated basic fraction contained **10** and the *N*-acetyl derivative of actinobolamine (**11**).

Registry No.—**1**, 25834-39-3; **2**, 25834-40-6; **3**, 25834-41-7; **4**, 78802-19-2; **5**, 25834-43-9; **6**, 25834-44-0; **7**, 25834-45-1; **8**, 25834-46-2; *N*-acetylactinobolin isopropylidine derivative, 25834-47-3; alanylactinobolone, 25834-48-4.

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(21) Merck Ag. Darmstadt HF 254 silicic acid distributed by Brinkmann Instruments.

(22) Studied by Mr. Chidambar L. Kulkarni.

(23) Studied by Mr. Fredrick J. Antosz.