Alanylactinobicyclone. An Application of Computer Techniques to Structure Elucidation[1]

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The structure proof of N-acetylalanylactinobicyclone (**1a**), a product isolated from the mild basic hydrolysis of N-acetylactinobolin, is described. Evidence was assembled which allowed the formulation of a partial structure, expressed as a set of five structural fragments, which accounted for all the atoms in the molecule. The set of fragments was used as input data for a computer program designed to generate all possible combinations of the fragments, *i.e.*, structures. A total of 11 possible structures were generated, of which eight were eliminated by chemical and spectral data on hand. An nmr study of the mono-O-acetate-d_6 derivative of N-acetylalanylactinobicyclone distinguished between the three remaining structural possibilities and permitted the assignment of 4-[N-(N'-acetylalanyl)]amino-3-methyl-9-hydroxy-2-oxabicyclo[3.3.1]nonan-7-one as **1a**.

As part of the investigation designed to reveal the molecular array of actinobolin,[2] a broad spectrum antibiotic possessing some activity against certain neoplastic diseases,[3] various degradation schemes were examined. An aqueous, acid-catalyzed degradation of the intact molecule, $C_{13}H_{20}N_2O_6$, led to the isolation of L-alanine, carbon dioxide, and actinobolamine,[3] a molecule containing an azabicyclic skeleton seemingly not present in the parent compound. Treatment of N-acetylactinobolin, $C_{15}H_{22}N_2O_7$, with 1 *N* ammonium hydroxide was found to produce 1 mol of carbon dioxide and two new products. Freeze drying of the hydrolysis mixture followed by a chromatographic separation gave N-acetylalanylactinobolone,[2] the initial basic degradation product of N-acetylactinobolin and the precursor of a second, more easily isolable compound, N-acetylalanylactinobicyclone (**1a**), whose proof of structure forms the subject of this manuscript.

The mass spectrum of N-acetylalanylactinobicyclone, a $C_{14}H_{22}N_2O_5$ molecule, displayed the appropriate molecular ion at *m/e* 298 and major peaks at *m/e* 86, 114 and 131. Examination of the nmr spectrum[4] (d_6-dimethyl sulfoxide) revealed a doublet at δ 5.42 (J = 3 Hz) with an integral amplitude equivalent to one hydrogen, which corresponded to the hydrogen of a secondary hydroxyl group. This assignment was verified by the immediate disappearance of the signal on addition of deuterium oxide to the sample.[5] Two one-proton doublets at δ 8.03 (J = 7 Hz) and 8.04 (J = 9 Hz) disappeared more slowly upon the addition of deuterium oxide, suggesting the presence of two secondary amide groups, each bonded to carbon bearing a single hydrogen. The region from δ 4.8 to 3.3 contained overlapping signals whose integral amplitude corresponded to five hydrogens, while the region from 3.2 to 2.0 contained a multiple pattern whose area also indicated five hydrogens. Higher field signals were attributed to an N-acetate methyl group (δ 1.87) and two secondary methyl groups (doublets at 1.22, J = 7 Hz, and 0.97, J = 6 Hz). The infrared spectrum (KBr) displayed peaks at 3550–3250 (broad, OH and amide NH), 1725–1625 (broad, ketone and amide C=O), and 1530 cm^{-1} (amide II band).

Acetylation of **1a** in acetic anhydride and pyridine led to the isolation of a mono-O-acetate **1b**, $C_{16}H_{24}N_2O_6$: ν_{max}^{KBr} 1715 (unstrained ketone C=O), 1740 (acetate C=O), 1640 (amide C=O), and 1540 cm^{-1} (amide II band), confirming the presence of a single hydroxyl group and a ketone function in **1a**. Support for the assignment of a ketone carbonyl in **1a** and the nature of the environment about it was disclosed by base-catalyzed deuterium exchange studies which were monitored by nmr. Addition of sodium deuterioxide to an nmr sample tube containing a solution of **1a** in deuterium oxide led to the loss of signals equivalent to four of the five hydrogens of the broad multiplet covering the region from δ 3.3 to 2.3.[6] After deuterium exchange only a one-hydrogen multiplet, centered at δ 2.55, remained. Compound **1a** was recovered unchanged (except for deuterium incorporation) following such treatment. Since the nmr spectrum of **1a** (or its derivatives) fails to display the singlet characteristic of the methyl group of a methyl ketone, these data indicate the presence of a ketone carbonyl flanked on either side by a methylene group, *i.e.*, $-CH_2COCH_2-$.

The nmr spectrum[4] (d_6-acetone) of the mono-O-acetate **1b** (listed in the Experimental Section) revealed five discrete one-hydrogen signals between δ 5.4 and 3.7 suggesting that each could be ascribed to hydrogen on carbon bearing a heteroatom. Three of the signals were readily assigned. The low field signal at δ 5.32 was assigned to the hydrogen on carbon bearing O-acetate. Multiplicity changes noted for signals at δ 4.75 and 4.08 on exchange of amide N–H for N–D established each of these signals to be due to a single hydrogen on carbon bearing secondary amide nitrogen.

The nature of the two remaining signals in this five-proton region was suggested by the observation that, of all the heteroatoms in the molecule, the functionality of only one, an oxygen atom, remained in doubt. Nmr evidence, then, infers the presence of an ether linkage, with the signals at δ 4.37 and 3.82 attributable to hydrogens on carbon bearing the ether oxygen. The chemical evidence described is also consistent with the incorporation of an ether linkage. Double-resonance studies[7] established that one and only one of these two

(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, 1969. (c) A portion of the work described was presented before the Medicinal Chemistry Division at the 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1968, MEDI 050.

(2) M. E. Munk, D. B. Nelson, F. J. Antosz, and D. L. Herald, Jr., *J. Amer. Chem. Soc.*, **90**, 1087 (1968).

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

(4) Reported in parts per million (δ) downfield of tetramethylsilane.

(5) O. L. Chapman and R. W. King, *ibid.*, **86**, 1256 (1964).

(6) Reported in parts per million (δ) downfield of 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt as an internal standard.

(7) Field-swept spin decoupling at 60 MHz.

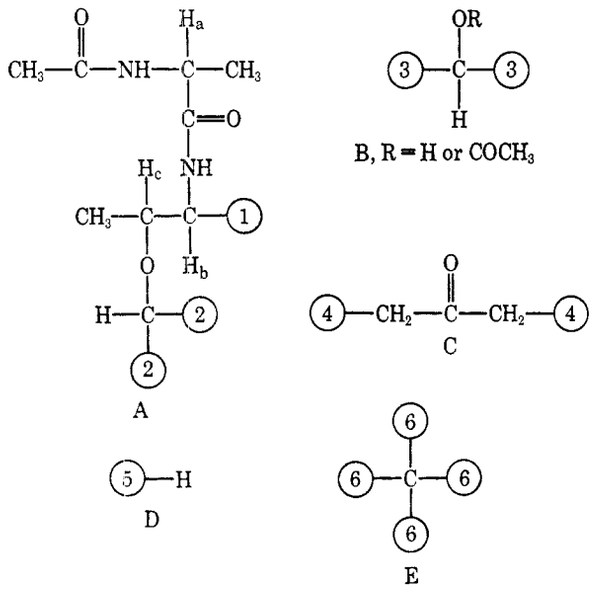
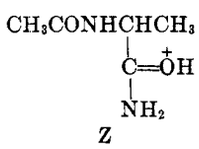


Figure 1.—Partial structure of N-acetylalanylactinobicyclone.

signals (δ 3.82) is coupled to a high field methyl signal, the doublet at 1.15. In concert, these data require the presence of a $CH_3\dot{C}HO\dot{C}H-$ unit.

At this stage of the problem, the evidence was collated and expressed as the partial structure shown in Figure 1. The presence of the N-acetylalanyl group in unit A, suggested by its occurrence in N-acetylactinobolin,[2] was supported by nmr and mass spectral evidence. After the exchange of hydrogen on amide nitrogen for deuterium, the nmr spectrum[4] of compound **1b** (d_6-acetone) disclosed the N-acetyl methyl group as a three-hydrogen singlet at δ 1.97. The alanyl moiety was represented by a methyl doublet at δ 1.35 (J = 7 Hz) which was shown to be spin coupled[7] to the methine hydrogen (H_a) quartet at 4.75 (J = 7 Hz). The mass spectrum peaks at m/e 86 ($CH_3\overset{+}{C}ONH{=}CHCH_3$), 114 [$CH_3CONHCH(CH_3)C{\equiv}O^+$], and 131 (Z)[8] further corroborated this assignment.



Z

Evidence linking the N-acetylalanyl group to the ether unit $CH_3\dot{C}HO\dot{C}H-$ through a CHNH unit, as shown in A, also derives from the nmr spectrum of **1b**. Based on evidence already presented, it follows that the N-acetylalanyl group is joined *via* nitrogen to carbon bearing a single hydrogen whose nmr signal appears at δ 4.08 (H_b). Only the nmr signal at δ 3.82 (H_c) shares a common coupling constant (J = 2.5 Hz) with the 4.08 signal, thus suggesting that the nitrogen atom in question must not only reside on a CH moiety contiguous to CH bearing the ether oxygen, but that the latter CH must bear a methyl group as well.

Fragment B (R = $COCH_3$) is simply the acetoxy unit derived from the secondary alcohol of **1a** and is represented in the nmr spectrum of **1b** by signals at δ 5.32 (1 H) and 2.10 (3 H). The methylene-flanked ketone is shown as fragment C. The hydrogens of the methylene groups must therefore provide four parts of a five-hydrogen nmr signal centered at δ 2.70. Fragments A through C account for all atoms of **1a**, except CH, and all of the nmr signals of **1b**, except a single hydrogen signal buried in the five-hydrogen signal at δ 2.70. These remaining atoms may be described in the least restrictive fashion as fragments D and E. Fragments A, B (R = H), C, D, and E, therefore, account for all the atoms of **1a** and, properly linked together, *must* reveal the structure of N-acetylalanylactinobicyclone.

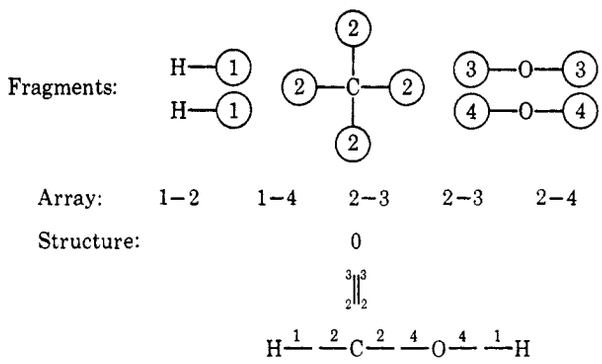


Figure 2.—Topological representation of formic acid.

A computer approach[9] has been designed which provides *all* the possible ways of joining a series of structural fragments, *i.e.*, all possible molecules consistent with the fragments, while sparing the investigator the tedium and uncertainty associated with the process of "manual combination." For a series of structural fragments such as those in Figure 1, the available bonding sites (half-bonds) may be represented by numbers (circled). A number pair then represents a full bond and an array of number pairs utilizing *all* of the numbers in the set represents a molecule. Using Huber's[10] terminology, the structural description is of the topological coding class, *i.e.*, a number-pair array shows "atom-to-atom connections in a compound with the atoms as nodes and the connections as branches of a network." As an example, the molecule formic acid can be formed from the five fragments shown in Figure 2. The number-pair array in Figure 2 is a representation of the formic acid molecule from which a recognizable structure can be reconstructed. After first assigning numbers to all bonding sites, the process of combining a series of structural fragments to form all possible molecules reduces to one of pairing a series of numbers in all possible arrays of number pairs, a task eminently suited to the computer.

Computer program CMBN accepts input in the form of a series of numbers representing the available bonding sites of a group of structural fragments and delivers an output which appears as all possible arrays of number pairs (structures). After each array of number pairs is generated and passes any tests which may be imposed, it is checked against other valid arrays so that each printed array is a unique set of number pairs. Five types of restrictions or controls are built into the program. One control is always in effect and

(8) The ion at m/e 131 could arise by double hydrogen rearrangement, a process of reasonable importance for cycloalkylamides (H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, Inc., San Francisco, Calif. 1967, pp 342–343).

(9) The computer program, written in FORTRAN IV and instructions for use are available from the authors (M. E. M.).

(10) M. L. Huber, *J. Chem. Doc.*, **5**, 4 (1965).

rejects arrays in which two bonding sites on the *same* atom are joined together; *e.g.*, if the atom in question were carbon, a carbene would be generated. This is achieved by assigning the *same* number to chemically equivalent bonding sites on the *same* atom and instructing the computer to reject arrays which contain number pairs of two identical numbers, *e.g.*, as 2–2. The four additional structure-limiting controls may be utilized as the available evidence warrants, by proper processing of the input data. First, the number of double and/or triple bonds may be specified. In this case only those arrays containing the specified multiplicity will appear. The second control allows any number of designated bonds to be forbidden. The printed arrays will not contain any of the forbidden number pairs. The third control allows a designated bond to be forbidden if another designated bond is formed (dual-bond restriction). Here those printed arrays will be rejected that contain *both bonds*. The fourth control requires designated bonding sites to bond only to one of two or one of three preselected sites. Only those arrays with *one* of the required bonds will appear in the output.

The CMBN computer program was employed to advantage in the solution of the structure of N-acetylalanylactinobicyclone at the stage of development indicated by Figure 1. Half-bond sites were designated by the number system shown. The input data instructed the computer to reject multimolecule answers, and all structures containing a methylene group bonded to amide nitrogen, a methyl ketone, a primary alcohol, or carbon–carbon double bonds. The absence of the latter group was deduced from a consideration of evidence derived from the nmr spectra of **1a** and **1b**. The exclusion of answers containing carbon–carbon double bonds was achieved in this case through the third control which rejected those answers where the number pairs 2–3, 2–6, or 3–6 appear twice. This dual-bond restriction requires, for example, that, if 2–3 appears in a solution, the presence of a second 2–3 number pair is forbidden. The presence of a methylene group bonded to amide nitrogen, a primary alcohol, and a methyl ketone was excluded *via* the second control by forbidding formation of the number pairs 1–5, 3–5, and 4–5, respectively. The third control was also used to reject multimolecule solutions arising from the appearance of the number pair 3–4 twice.

The output appeared as eleven number-pair arrays (Table I). Of the eleven possible structures, the infrared data for **1b** (ν_{max}^{KBr} 1715 cm^{-1}, unstrained ketone C=O) excluded four structures (**4**, **5**, **6**, **7**) which contained cyclobutanone rings and three structures (**8**, **9**, **10**) which contained cyclopentanone rings.[11] An additional structure **11**, which contained a cyclopropanol ring fused to an eight-membered cyclic ether, was also rejected. Such a cyclopropanol derivative was inconsistent with the acid and base stability of **1a**.[12] A total of three possible structures remained (**1**, **2**, and **3**), all of which feature a substituted cyclohexanone ring.

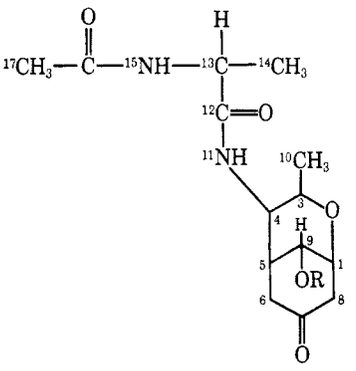
Thus the problem of assigning a structure to N-acetylalanylactinobicyclone was reduced to a choice between **1**, **2**, and **3**. The nmr spectrum[4] (d_6-acetone) of the O-acetate derivative was the focal point of attention in making that choice. The signal at δ 5.32 for hydrogen on carbon bearing the O-acetate appeared as a triplet (J = 4 Hz), suggesting this hydrogen was equally coupled to two other hydrogens on adjacent carbon atoms. On this basis, structure **1b**, with a single hydrogen at each of the adjacent bridgehead positions, C-1 and C-5, seemed the most attractive candidate, since in structures **2b** and **3b** there are a total of *three* hydrogens on adjacent carbon. However, the possibility that in structure **2b** or **3b** the vicinal coupling constant involving *one* of the geminal hydrogens approached zero could not be excluded. Therefore, the multiplicity of this signal *per se* failed to provide incontrovertible evidence for structure **1b**.

The relationship of the hydrogen on carbon bearing the O-acetate to those hydrogens on carbon flanking the ketone carbonyl in structure **1b** on the one hand and structures **2b** and **3b** on the other hand suggested the

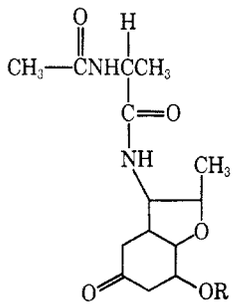
TABLE I

COMPUTER OUTPUT OF POSSIBLE STRUCTURES FOR N-ACETYLALANYLACTINOBICYCLONE

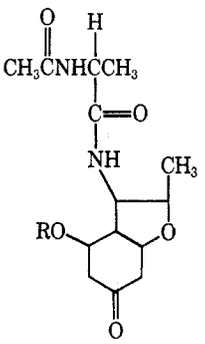
Structure number	Number-pair array					
1	1–6	2–3	2–4	3–6	4–6	5–6
2	1–6	2–3	2–6	3–4	4–6	5–6
3	1–6	2–4	2–6	3–4	3–6	5–6
4	1–2	2–3	3–6	4–6	4–6	5–6
5	1–3	2–3	2–6	4–6	4–6	5–6
6	1–3	2–5	2–6	3–6	4–6	4–6
7	1–6	2–3	2–5	3–6	4–6	4–6
8	1–2	2–6	3–4	3–6	4–6	5–6
9	1–3	2–4	2–6	3–6	4–6	5–6
10	1–6	2–5	2–6	3–4	3–6	4–6
11	1–4	2–3	2–6	3–6	4–6	5–6



1a, R = H
b, R = $COCH_3$



2a, R = H
b, R = $COCH_3$



3a, R = H
b, R = $COCH_3$

(11) See K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, Inc., San Francisco, Calif., 1962, p 42.

(12) The susceptibility of cyclopropanols to ring opening under acidic and basic conditions has been described [C. H. DePuy, F. W. Breitberl, and K. R. DeBruin, *J. Amer. Chem. Soc.*, **88**, 3347 (1966)].

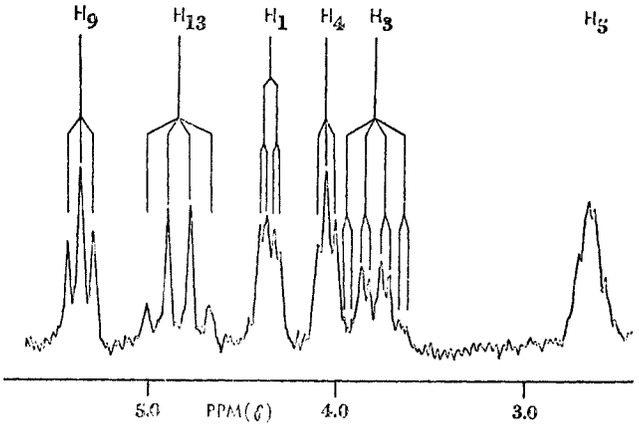


Figure 3.—Nmr spectrum of the hexadeuterio-O-acetate of N-acetylalanylactinobicyclone.

design of an informative experiment. Exchange of the four acidic hydrogens for deuterium on carbon flanking the ketone carbonyl should result in a decrease in the multiplicity of the signal for the hydrogen on carbon bearing the O-acetate if structure **2b** or **3b** were correct; no change in multiplicity would be expected for structure **1b**.

The hexadeuterio-O-acetate of N-acetylalanylactinobicyclone (exchange of acidic hydrogens on carbon α to the ketone carbonyl and the amide nitrogens) was prepared by acetylation (acetic anhydride in dry pyridine) of heptadeuterio-**1a**, which in turn was obtained by twice freeze drying a deuterium oxide solution of **1a** containing potassium carbonate. The nmr spectrum[4] of deuteriated **1b** was run in *d*-chloroform with an overlay of a dilute solution of potassium carbonate in deuterium oxide. The nmr sample tube was gently shaken to remove residual acidic hydrogen and, when the integrated area of the multiplet at δ 2.67 corresponded to a single proton (the nmr of compound **1b** displays a five-proton multiplet in the δ 2.7 region when determined in *d*-chloroform), the complete spectrum was traced (Figure 3).

The triplet nature of the low field signal (at δ 5.38) *remained unchanged*, although it appeared somewhat better defined than in the spectrum of nondeuteriated **1b**. This evidence requires structure **1a** for N-acetylalanylactinobicyclone. The complete nmr spectrum of hexadeuterio-**1b**, together with spin-decoupling experiments (Table II), provided strong verification of the structure assigned.

The multiplicity and coupling constant ($J_{9,1}$, $J_{9,5}$ = 4 Hz) of the signal for H_9 is in the range expected for the one-carbon bridge proton of a 9-substituted bicyclo[3.3.1]nonane system.[13] Irradiation of the H_5 signal at δ 2.67 resulted in the collapse of the H_9 triplet at 5.38 to a doublet, $J_{9,1}$ = 4 Hz.[14] The presence of deuterium at C-8 reduced the H_1 signal at δ 4.40 from a multiplet (in the absence of deuterium) to a doublet of doublets; $J_{1,9}$ = 4 Hz and $J_{1,5}$ = 2 Hz. The magnitude of the latter coupling constant is comparable with long range coupling observed in other rigid systems possessing the

(13) H. O. House, H. C. Muller, C. G. Pitt, and P. P. Wickham [*J. Org. Chem.*, **28**, 2407 (1963)] report the H_9 signal for 3-methyl-3-azabicyclo[3.3.1]-nonan-9-ol as a triplet, J = 3 Hz.

(14) The chemical shift difference between the H_9 and H_1 signals is such (~60 Hz) that effective spin decoupling is not possible with the Varian Associates Model V-6058A spin decoupler.

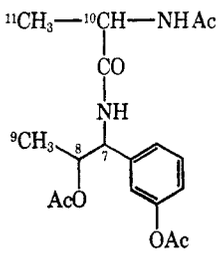
TABLE II
SPIN-DECOUPLING STUDIES ON HEXADEUTERIO-**1b**[a,b]

Signal irradiated (δ, multiplicity, J in Hz)	Signal observed (δ)	Multiplicity change
H-13 (4.90, q, 7)	H-14 (1.41)	d → s
H-14 (1.41, d, 7)	H-13 (4.90)	q → s
H-3 (3.83, q of d, 6, 2.5)	H-10 (1.05)	d → s
H-10 (1.05, d, 6)	H-3 (3.83)	q of d → d, J = 2.5 Hz
H-5 (2.67, m)	H-9 (5.38)	t → d, J = 4 Hz
H-5 (2.67)	H-1 (4.40)	d of d → d, J = 4 Hz
H-5 (2.67)	H-4 (4.08)	t → d, J = 2.5 Hz
H-9 (5.38, t, 4)	H-5 (2.67)	m → ~ t[c]
H-1 (4.40, d of d, 4, 2)	H-5 (2.67)	m → ~ d of d[c]
H-4 (4.08, t, 2.5)	H-5 (2.67)	m → ~ d of d[c]

[a] Field-swept decoupling at 60 MHz. [b] In $CDCl_3$ *vs* TMS (δ 0.0). [c] Coupling constants difficult to measure accurately.

required "W" arrangement through four bonds.[15] The observed change in the multiplicity of the H_1 signal to a doublet, $J_{1,9}$ = 4 Hz, upon irradiation of the H_5 signal confirmed the long range coupling constant, $J_{1,5}$ = 2 Hz. Irradiation of the H_5 signal resulted in the decoupling of a third signal in the spectrum; the H_4 triplet, $J_{4,3}$ = $J_{4,5}$ = 2.5 Hz, at δ 4.08 collapsed to a doublet, J = 2.5 Hz. Additional decoupling confirmed the previously demonstrated vicinal relationship of H_{13} and H_{14} ($J_{13,14}$ = 7 Hz) and H_3 and H_{10} ($J_{3,10}$ = 6 Hz). Thus the chemical and physical properties of N-acetylalanylactinobicyclone are accommodated only by structure **1a**.

The perchloric acid catalyzed acetylation of **1a** gave rise to the previously characterized *meta*-substituted phenyl acetate **12**, the compound isolated from the perchloric acid catalyzed acetylation of N-acetylalanylactinobolone.[2] This evidence supports the 1–3 relationship between the potential side chain at C-5 and the ketone carbonyl group of **1a**.



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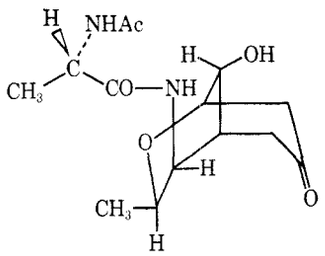
Several pieces of information suggest, but do not require, the absolute configuration and conformation (in solution) of N-acetylalanylactinobicyclone. The configuration at C-13 is established as *S* by the isolation of L-alanine upon vigorous acid hydrolysis of **1a**. Permanganate oxidation of both actinobolin[2] and its acid degradation product, actinobolamine[3] (**13**), gave rise to L-threonine. An examination of the structure of actinobolin[2] suggests that it is equally likely that base-induced cleavage of N-acetylactinobolin would proceed with retention of configurational integrity at C-3 and C-4 (of actinobolin); therefore C-3 and C-4 of **1a** may be assigned as *R* and *R*, respectively.[16] Basic conditions

(15) D. Gagnaire, E. Payo-Subiza, and A. Rousseau, "Nuclear Magnetic Resonance in Chemistry," B. Pease, Ed., Academic Press Inc., New York, N. Y., 1965, pp 165–171.

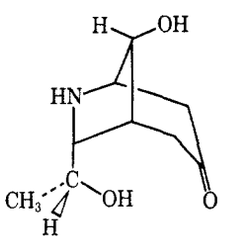
(16) The formation of **1a** from N-acetylalanylactinobolone[2] and actinobolamine from actinobolin is discussed in a manuscript in preparation, D. B. Nelson and M. E. Munk. Briefly **1a** derives from intramolecular conjugate addition of the side-chain OH group to the α,β-unsaturated ketone linkage derived from N-acetylalanylactinobolone by loss of water.

would, likewise, not be expected to effect the stereochemical integrity of C-10 of actinobolin, which has recently been assigned the *R* configuration;[17] therefore C-5 of **1a** may be assigned as *R*. These assignments *fix* the configuration of C-1 as *S*.

Two observations favor the placement of the hydroxyl group at C-9 in a position *syn* to the keto bridge, *i.e.*, in the *R* configuration;[18] the hydroxyl group at C-8 of actinobolamine (**13**) is *syn* to the keto bridge,[16] and the improved resolution observed for the H_9 triplet (nmr) upon exchange of hydrogen for deuterium at C-6 and C-8 is compatible with the elimination of small "W" coupling[15] between H_9 and the *endo* hydrogens at C-6 and C-8. The twin-chair conformation shown for **1a** is consistent with this latter observation and is reported to be the preferred geometry of bicyclo[3.3.1]-nonane,[19] especially in the absence of *endo* substituents at positions 3 and 7.[20] Although conceivable, it does



1a



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not appear likely that intramolecular hydrogen bonding between the hydroxyl group at C-9 and the ketone carbonyl (OH···O=C) would significantly alter the preference for the twin-chair in favor of the chair-boat conformation.[21]

In conclusion, it should be noted that the computer technique described in this paper harmonizes with and expedites the total process of structure elucidation. At the last stages of the process the investigator has the assurance that *all structures* consistent with the chemical and physical properties of the molecule have been considered. In addition, access to the program provides a convenient and expeditious method of generating at an earlier stage—earlier than the investigator would ordinarily contemplate—the spectrum of structures implicated by the evidence available at that time. The examination of these possible structures can afford an invaluable guide to the design of those incisive experiments necessary to identify the correct structure.

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-4 mass spectrometer using a heated direct inlet system, ionizing current of 19μA, and ionizing energy of 70 eV. Solvent systems, adsorbant, and visualization methods for tlc are listed where used. Microanalyses were performed by Midwest Microlab, Inc., Indianapolis, Ind.

Preparation of N-Acetylalanylactinobicyclone (1a).—A solution of 2.043 g (5.97 mmol) of N-acetylactinobolin in 50 ml of 5 *N* ammonium hydroxide was refluxed for 55 min. The solution was freeze dried and the resulting solid was adsorbed onto silicic acid,[22] dry loaded into a column containing 60 g of silicic acid[22] and eluted with ethyl acetate containing increasing amounts of ethyl alcohol. Elution with ethyl acetate–ethyl alcohol (25:4, v/v) gave a homogeneous product band containing the bulk of the weight. Continued elution gave cuts which also contained a second, less mobile product. Rechromatography of the mixed product cuts resulted in isolation of both product zones. The less mobile product was crystallized from acetone to give 109 mg of N-acetylalanylactinobolone.[2] Crystallization of the more mobile product from ethyl acetate gave 1.295 g (73%) of N-acetylalanylactinobicyclone (**1a**), mp 136–138°. Recrystallization from ethyl acetate followed by extensive vacuum drying gave an analytical sample: mp 137–138°; $[\alpha]^{25}D$ −7.5° (*c* 4.5, CH_3OH); ν_{max}^{KBr} 3550–3250 cm^{-1} (broad, OH and amide NH), 3070 cm^{-1} (amide NH), 1725–1625 cm^{-1} (ketone and amide C=O), 1530 cm^{-1} (amide II); ORD (*c* 2.6, CH_3OH) positive Cotton curve (27°), $[\phi]_{600}$ −13°, $[\phi]_{450}$ −39°, $[\phi]_{344}$ −65°, $[\phi]_{311}$ +147°, $[\phi]_{276}$ −1170°; nmr (*d*$_6$DMSO), see discussion; nmr (D_2O) δ 4.7–3.7 (5 H, m, hydrogen on carbon bearing heteroatoms), 3.3–2.3 (5 H, m, methine and α to C=O methylene), 2.05 (3 H, s, $NCOCH_3$), 1.39 (3 H, d, J = 7 Hz, $CHCH_3$), 1.10 (3 H, d, J = 6 Hz, $CHCH_3$); mass spectrum *m/e* (rel intensity) 298 (10), 280 (2), 254 (5), 170 (13), 168 (47), 150 (20), 141 (21), 131 (56), 114 (71), 87 (100), 86 (91).

Anal. Calcd for $C_{14}H_{22}N_2O_5$: C, 56.36; H, 7.43; N, 9.39; O, 26.82; mol wt, 298. Found: C, 56.33; H, 7.62; N, 8.93; O, 27.04; mol wt, 298 (mass spectrum).

Acetylation of 1a. Preparation of the Mono-O-acetate 1b.—A solution of 450 mg (1.51 mmol) of **1a** in 3 ml of acetic anhydride and 3 ml of dry pyridine was stirred at room temperature for 12 hr. Removal of solvent *via* high vacuum left a gum which was crystallized from chloroform–methylcyclohexane to give 473 mg (92%) of **1b**, mp 177–179°. Recrystallization gave analytically pure material: mp 179–180°; $[\alpha]^{25}D$ −22.9° (*c* 4.5, MeOH); ν_{max}^{KBr} 3400–3200, 3070 (amide NH), 1745 (acetate C=O), 1715 (unstrained ketone C=O), 1640 (amide C=O), 1535 cm^{-1} (amide II); ORD (*c* 2.5, MeOH) positive cotton curve (27°), $[\phi]_{600}$ − 73°, $[\phi]_{450}$ − 142°, $[\phi]_{356}$ − 210°, $[\phi]_{314}$ + 47°, $[\phi]_{270}$ −1320°; nmr (*d*$_6$-acetone) δ 7.95 (1 H, d, J = 9 Hz, NH), 7.49 (1 H, d, J = 7 Hz, NH), 5.32 (1 H, t, J = 4 Hz, H-9), 4.75 (1 H, quintet, J = 7 Hz, H-13), 4.37 (1 H, m, H-1), 4.8 (1 H, d of t, J = 9 and 2.5 Hz, H-4), 3.82 (1 H, q of d, J = 7 and 2.5 Hz, H-3), 3.1–2.2 (5 H, m, H-5, H-6, H-8), 2.10 (3 H, s, $OCOCH_3$), 1.97 (3 H, s, $NCOCH_3$), 1.35 (3 H, d, J = 7 Hz, H-14), 1.15 (3 H, d, J = 6 Hz, H-10); mass spectrum *m/e* (rel intensity) 341 (M + 1, 100), 340 (66), 228 (72), 150 (82), 131 (74), 114 (87), 87 (91), 86 (91).

Anal. Calcd for $C_{16}H_{24}N_2O_6$: C, 56.45; H, 7.11; N, 8.23; mol wt, 340. Found: C, 56.74; H, 7.38; N, 8.20; mol wt, 340 (mass spectrum M + 1, 341).

To a solution of 15 mg of **1b** in 1 ml of methanol was added 0.1 ml of 0.1 *N* sodium methoxide; the resulting solution was stirred at room temperature for 30 min. The solution was passed through a column containing 3 ml of methanol-washed Amberlite IRA-120 cation-exchange resin (proton form). A tlc examination of the film left after removal of solvent under reduced pressure demonstrated the presence of **1a** as the major product. The following systems were used and were visualized *via* H_2SO_4 charring (ad-

(17) Presented before the Division of Organic Chemistry of the American Chemical Society, Atlantic City, N. J., Sept 1968, ORGN 22.

(18) The C-5 position of actinobolin, from which C-9 of **1a** derives, has been designated as an *R* center.[17]

(19) W. A. C. Brown, G. Eglinton, J.Martin, W. Parker, and G. A. Sim, *Proc. Chem. Soc.*, 57 (1964).

(20) R. A. Appleton, C. Egan, J. M. Evens, S. H. Graham, and J. R. Dixon, *J. Chem. Soc., C*, 1110 (1968).

(21) R. D. Stolow [*J. Amer. Chem. Soc.*, **84**, 686 (1962)] reports that no evidence of intramolecular hydrogen bonding could be detected by infrared spectroscopy in 4-hydroxy- and 4-phenyl-4-hydroxycyclohexanone. J. Rigandy and P. Courtot [*Compt. Rend.*, **248**, 3016 (1959)] report the appearance of both unassociated OH stretching and OH···O=C stretching frequencies ($\Delta\nu$ 20 cm^{-1}) in the high dilution infrared spectrum of 4-hydroxycycloheptanone.

(22) Bio-Sil A(100-200 mesh) silicic acid purchased from Bio-Rad Laboratories.

sorbant, developing solvent): Merck HF 254,[23] acetone, Adsorbosil-3,[24] ethyl acetate–ethyl alcohol, 2:1, v/v, Adsorbosil-3, benzene–methanol, 5:1, v/v, Adsorbosil-3, acetone, Bio-Sil A,[25] 1-butanol–water–acetic acid, 4:5:1, v/v/v. Preparative tlc (Merck HF 254, acetone) of the remaining deacylated product gave a white solid which after crystallization from ethyl acetate revealed an ir spectrum identical with that of **1a**.

Acetylation of 1a. Preparation of the Aromatic O-Acetate (12).—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 511 mg (1.71 mmol) of **1a** and 7 ml of acetic anhydride. The slurry was stirred magnetically at 0° for 30 min under nitrogen flow. Four drops of perchloric acid were the nadded at the rate of 1 drop/4 min. The solution was allowed to come to room temperature over a period of 30 min, and poured onto ice and the water layer extracted three times with 20-ml portions of dichloromethane. The combined dichloromethane extracts were back-washed with 5% sodium bicarbonate solution and water and then dried over anhydrous magnesium sulfate. Volatile solvent was removed under reduced pressure. Further solvent removal under high vacuum left a crystalline mass which was triturated with cold ethyl acetate and filtered. A second crystal crop derived from the filtrate brought the crude yield of **12** to 301 mg (48%), mp 140–143°. Recrystallization from ethyl acetate gave a pure material, mp 146–147°, whose physical and spectral properties were identical with those displayed by the aromatic O-acetate isolated after treatment of N-acetylalanylactinobolone under the same reaction conditions.[2]

Deuterium Exchange. Preparation of Hexadeuterio-1b.—To a solution of 10 ml of deuterium oxide and 200 mg of potassium carbonate was added 360 mg of **1a**. The reaction flask was sealed, allowed to stir at room temperature for 18 hr and then freeze dried. The freeze-dried solid was taken up in hot ethyl acetate and filtered. After removal of solvent under reduced pressure and high vacuum the filtrate material was resubjected to the deuterium exchange conditions outlined above. After the second deuterium exchange, the ethyl acetate soluble material was crystallized from ethyl acetate to give 128 mg of heptadeuterio-**1a**. The nmr spectrum (D_2O) of this material shows a single methine hydrogen at δ 2.55.

A solution of 100 mg of heptadeuterio-**1a** (prepared as above) in 2 ml of dry pyridine and 2 ml of acetic anhydride was stirred, under a nitrogen blanket, at room temperature for 16 hr. Removal of the solvent under high vacuum left a clear film which was freeze dried from deuterium oxide, taken up in 0.4 ml $CDCl_3$, and loaded into an nmr probe. The nmr spectrum indicated a small amount of hydrogen exchange in the acetylation process. An overlay of deuterium oxide–potassium carbonate solution was added to the sample tube and the tube was shaken for 5 min. The nmr spectrum indicated a single hydrogen signal centered at δ 2.67. To quench exchange the deuterium oxide layer was removed from the tube, additional deuterium oxide was added, shaken, and removed before proceeding with additional nmr spectra of hexadeuterio-**1b**.

Acid Hydrolysis of 1a. Isolation of L-Alanine.[26]—A solution of 500 mg (1.68 mmol) of **1a** in 4 ml of 4 *N* sulfuric acid was warmed on a steam bath for 15 hr. The reaction solution was passed through a column containing 35 ml of AG 21-K anion-exchange resin (hydroxide form). The column was eluted with water until the eluents were neutral. The column was next eluted with 10% acetic acid. These eluents were freeze dried to give a fluffy powder which on crystallization from aqueous ethanol gave 103 mg (1.15 mmol) of white crystalline solid: $[\alpha]^{25}D$ +2.6° (*c* 6.4, H_2O) [lit.[27] for L-alanine $[\alpha]^{25}D$ +2.7 (*c* 10.5, H_2O)]. Its nmr spectrum (D_2O) was identical with that of alanine and its plain, positive ORD curve (*c* 6.4, H_2O) was superimposable on the ORD curve of an authentic sample of L-alanine.

Freeze drying of the water eluent gave an amorphous solid. A solution composed of this solid in 10 ml of ethanol and 0.5 ml of acetic anhydride was stirred for 12 hr at room temperature. Solvent was removed *via* high vacuum to give a residue which was crystallized from ethyl alcohol–methylcyclohexane to give 153 mg (0.67 mmol) of N-acetylactinobolamine,[3] mp 191–193°.

Registry No.—**1a**, 21902-56-7; **1b**, 21902-57-8; hexadeuterio-**1b**, 21955-11-3.

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

(24) Adsorbosil-3 silicic acid with 10% binder purchased from Applied Science.

(25) Bio-Sil A (10–20 μ) silicic acid with 5% binder purchased from Bio-Rad Laboratories.

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

(27) "Handbook of Chemistry and Physics," 43rd ed, Chemical Rubber Publishing Co., Cleveland, Ohio, 1961–1962, p 1764.