TABLE III
PRODUCTS FROM HYDROLYTIC CLEAVAGE OF 7

Aryl group	Registry no.	X	Y	Mp, °C	% C Calcd	% C Found	% H Calcd	% H Found	% N Calcd	% N Found	% yield
p-Chlorophenyl	16047-82-8	Cl	Cl	251–254 dec	44.93	45.29	5.03	5.24	5.82	5.79	69
p-Tolyl	16047-83-9	Cl	Cl	234–237 dec	54.56	55.16	6.87	7.00	6.36	6.44	87
p-Nitrophenyl	16047-84-0	Cl	Cl	148–150	43.04	43.11	4.82	5.03	11.15	11.15	62
Phenyl[a]		$^1/_2C_2O_4$	OH	194–195							
p-Tolyl	16052-65-6	HC_2O_4	OH	161–162	56.46	56.34	6.71	6.82	5.48	5.41	72
p-Chlorophenyl	16047-85-1	$^1/_2C_2O_4$	OH	212–214[b]	52.29	52.14	5.27	5.36	6.10	6.07	23

[a] See Table I, footnote *a*. [b] Another form melts at 163–164°.

was stirred at 0–5°, a solution of 7.5 g (0.10 mol) of sodium nitrite in 50 ml of water was added dropwise. The ether layer was separated, washed with 5% $NaHCO_3$ and water, and dried over anhydrous magnesium sulfate. An equal volume of benzene was added and the mixture was refluxed for 12 hr. During this time, the 6-aryltetrahydrooxazin-2-one crystallized in an extremely pure condition, as evidenced by the fact that recrystallization did not raise the melting point. Evaporation of the mother liquors yielded an additional, less pure sample which could be recrystallized from benzene.

Acidic Hydrolysis of 6-Aryltetrahydro-1,3-oxazin-2-ones.—A sample of **7** was covered with concentrated HCl and allowed to stand for 12 hr at room temperature. An exception was 6-(*p*-nitrophenyl)tetrahydro-1,3-oxazin-2-one, which requires steam bath temperature. The reaction mixture was then evaporated to dryness, leaving the 3-chloro-3-arylpropylamine hydrochloride (**8**) as a crude solid which could be recrystallized from ethanol or ethanol–benzene.

Basic Hydrolysis of 6-Aryltetrahydro-1,3-oxazin-2-ones.—A solution of **7** in alcoholic KOH was prepared, using 2 ml of 6 *N* KOH in ethanol for each millimole of **7**. The reaction mixture was refluxed for 5 hr, diluted with an equal volume of water, and acidified to pH 2 with 6 *N* HCl, during which CO_2 was evolved. The solution was then extracted with ether to remove nonbasic impurities, the water layer was made basic with 6 *N* NaOH, and the liberated organic base was extracted with ether. The ether extract was dried over anhydrous magnesium sulfate followed by removal of ether, leaving the 3-hydroxy-3-arylpropylamine (**9**) as an oil. This was isolated as the oxalate or acid oxalate by precipitating it from ethanol solution by adding a saturated solution of oxalic acid in ethanol. The salt was recrystallized from aqueous ethanol.

Acknowledgment.—The authors are grateful to Research Corporation for partial support of this project in the form of a Frederick Gardner Cottrell grant in aid.

Anisomycin. III.[1] Conformational Studies of the Pyrrolidine Ring

K. BUTLER

Chas Pfizer Medical Research Laboratories, Groton, Connecticut

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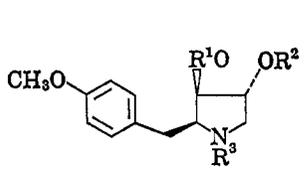
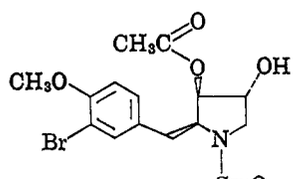
The first structural studies of anisomycin were carried out by classical chemical methods[2] and lead to the proposal of an all-*trans* relationship for the substituents on the pyrrolidine ring. However, recent X-ray crystallographic studies on N-acetylbromoanisomycin (**14**) have shown that the original stereochemical assignments were in error.[3] The correct structure for anisomycin (**1**) is shown above.





14

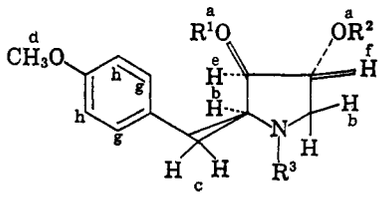
1, $R^1 = CH_3CO$; $R^2 = R^3 = H$
2, $R^2 = CH_3CO$; $R^1 = R^3 = H$
3, $R^1 = R^2 = R^3 = H$
4, $R^1 = CH_3CO$; $R^2 = H$; $R^3 = C_6H_5CH_2OCO-$
5, $R^1 = R^3 = CH_3CO$; $R^2 = H$
7, $R^1 = R^2 = R^3 = CH_3CO$
12, $R^1 = R^2 = H$; $R^3 = CH_3CO$
13, $R^1 = R^2 = CH_3CO$; $R^3 = H$

In the course of the initial investigation of anisomycin, attempts were made to use nuclear magnetic resonance spectra to elucidate the stereochemistry but without success. However, now that we know the stereochemistry of anisomycin, it is possible to explain some of the anomalies observed in the nmr spectra and to shed some light on the conformational properties of the pyrrolidine ring.

The proton magnetic resonance spectrum of anisomycin (**1**) in $CDCl_3$–D_2O and the assignments for chemical shifts are shown in Table I. Signals for the aromatic protons h and g indicate an A,B^2 pattern typical of a 1,4-disubstituted benzene ring, and the occurrence of a singlet at 3.78 ppm (methoxy) substantiates our previous observations that anisomycin contains a *p*-methoxyphenyl group. A single peak observed at 2.12 ppm is due to the methyl (a) of the acetate group. The benzyl methylene of anisomycin is adjacent to an asymmetric carbon atom (C-2), so that we should expect the methylene function to give rise to two separate and distinct nmr signals for the two diastereomeric methylene protons. Each signal should be split by the neighboring hydrogen atom at C-2 to produce a quartet of peaks due to the benzyl methylene, and this is observed at about 3.4 ppm, but the quartet is complicated further by splitting which is not so readily assigned. The diastereoisomerism of the benzyl methylene protons should be independent of conformation, for no matter how rapid the rate of torsion around the C–C bond, the environments of the

(1) Anisomycin. II: K. Butler, *J. Org. Chem.*, **31**, 317 (1966).
(2) J. J. Beereboom, K. Butler, F. C. Pennington, and I. A. Solomons, *J. Org. Chem.*, **30**, 2334 (1965).
(3) J. P. Shaefer and P. J. Wheatley, personal communication.

TABLE I



	a	b	c	d	e	f	g	h	R³
1 (R¹ = acetyl; R² = H)	2.12 (s) 3 H	*Ca.* 2.7 (m) 3 H	3.4 (q) 2 H (J = 12.0; 6.2 cps)	3.78 (s) 3 H	4.75 (d) 1 H (J = 4.05 cps)	*Ca* 2.7 (m) 1 H (J = 6.0; 3.5 cps)	6.83 (d) 2 H J = 8.6 cps	7.15 (d) 2 H J = 8.6 cps	...
1[a]					4.75 (q) 1 H J = 4.05; 0.7 cps	4.13 (oct) 1 H J = 6.0; 3.5 and 0.7 cps			
2 (R¹ = H; R² = acetyl)	2.05 (s) 3 H	*Ca.* 2.82 (m) 3 H	3.45 (q) 2 H J = 12.5; 6.0 cps	3.78 (s) 3 H	3.87 (d) 1 H J = 3.5 cps	4.94 (q) 1 H J = 6.5; 3.0 cps	6.85 (d) 2 H J = 9.0 cps	7.25 (d) 2 H J = 9.0 cps	...
4 (R¹ = acetyl; R = H)	2.05 (s) 3 H	*Ca.* 3.0 (m) 3 H	3.50 (d) 2 H J = 5.0 cps	3.75 (s) 3 H	4.94 (q) 1 H J = 6.0; 5.0 cps	4.04 (m) 1 H ...	6.76 (d) 2 H J = 8.5 cps	7.06 (d) 2 H J = 8.5 cps	5.13 (s) 2 H benzylic CH_2 7.35 (s) 5 H phenyl

[a] Additional coupling of the protons e and f was not always observed.

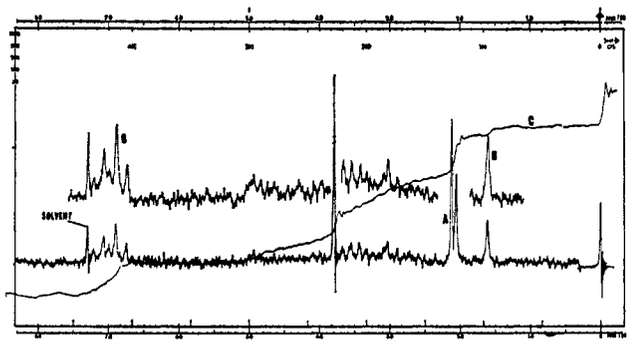


Figure 1.

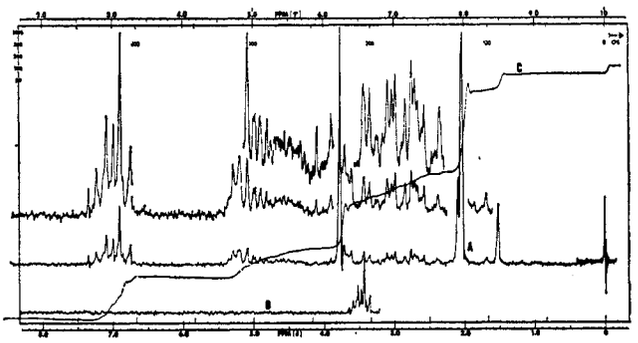
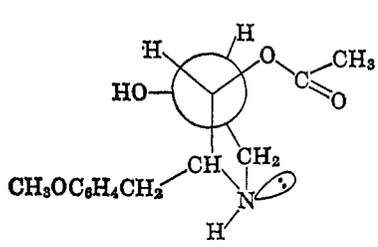


Figure 2.

two protons should always remain different. However, spectra of the benzyloxycarbonyl derivative (**4**) of anisomycin appear to have only a doublet rather than the quadruplet expected for the benzyl methylene. We have no explanation for this phenomenon.

The nmr data for the hydrogen atoms of the pyrrolidine ring are more difficult to interpret, but reveal much information concerning the conformation of anisomycin. We expected the signal for the proton e (Table I) to be split by f and by b to give a quartet, but the proton–proton coupling for e and f was very small (0.6–0.8 cps), and in fact was not observed in many spectra so that the signal for e had the appearance of a simple doublet. The hydrogen atom f gave rise to a quartet, whereas a much more complicated pattern would have been expected if there had been interactions between all of the neighboring hydrogen atoms. These data suggest that anisomycin has a rigid conformation in which the pyrrolidine ring is twisted so that the dihedral angle for the protons f and e is close to 90°. The H–H coupling constants for e and f would then be very small,[4] but there would still be splitting by protons at the α positions of the pyrrolidine ring. Thus, e is coupled with one proton (J = 4.05 cps) and f is coupled to two protons (J = 3.5 and 6.0 cps). In this rigid arrangement, the *p*-methoxy benzyl group is quasi-equatorial, and the C-3, C-4 substituents on the

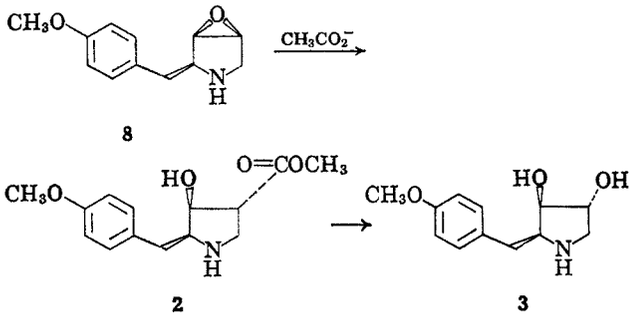


pyrrolidine ring assume a quasi-axial conformation. This would seem to be the least preferred conformation unless there is some structural feature present to stabilize it. In this case it is clear that the conformer is stabilized by interaction of the pyrrolidine nitrogen atom with the carbonyl of the acetoxyl function. The lone pair of electrons of the nitrogen atom appear to be attracted toward the carbon of the carbonyl group and the transannular interaction causes the pyrrolidine ring to buckle. This field effect lowers the electron density about the nitrogen atom and results in a reduced affinity for hydrogen ions. Thus aniso-

(4) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); M. Karplus and D. H. Anderson, *ibid.*, **30**, 6 (1959); H. Conroy, *Advan. Org. Chem.*, **2**, 265 (1960).

mycin is a weak base (pK 7.75), but deacetylanisomycin (**3**) in which such transannular interactions cannot occur, is much more basic (pK 8.8). An alternate hypothesis invokes a hydrogen bond between nitrogen and the carbonyl oxygen to stabilize the rigid anisomycin conformer. However, a hydrogen bond is an incipient proton transfer, which should leave a slight negative charge on the nitrogen atom and make anisomycin *more* basic, but this is contrary to the experimental facts. Furthermore, the infrared spectrum of anisomycin shows a strong N–H signal (2.99 μ) and no evidence of hydrogen bonding.

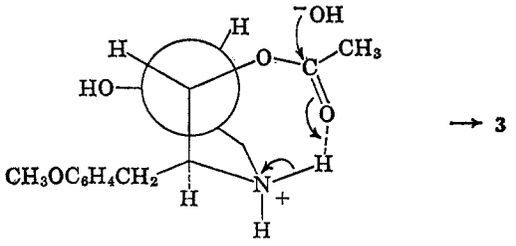
Isoanisomycin (**2**) is obtained from the epoxide **8** on treatment with acetic acid.[2] Isoanisomycin and anisomycin have the same stereochemistry and differ only with respect to the position of the acetoxyl group. Both compounds give the same deacetyl derivative **3** on hydrolysis.



The pyrrolidine ring of isoanisomycin (**2**) would be expected to have the same conformation as in anisomycin. Attraction between the pyrrolidine nitrogen atom and the acetoxyl grouping at C-4 would cause the C_3–C_4 bond to twist such that the hydrogen atoms e and f are at right angles to each other and this effect is seen in the nmr spectrum of isoanisomycin **2** (Table I). The benzylic hydrogens still show as a quartet, though partly obscured in the nondeuterated sample. The hydrogen e at C-3 of the pyrrolidine ring has shifted upfield close to the methoxyl signal, but still shows a simple doublet due to splitting by the hydrogen b at C-2. The hydrogen f at C-4 has moved downfield to 4.94 ppm and gives rise to a quartet due to splitting by the two diastereomeric hydrogens at C-5.

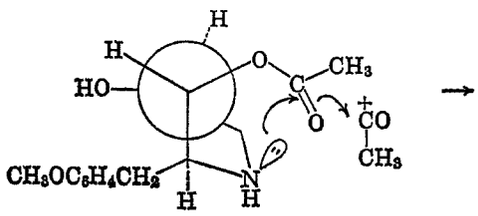
Isoanisomycin is a weak base (pK 7.93), but it is somewhat more basic than anisomycin (pK 7.75). This suggests that the transannular interactions for isoanisomycin are not so strong as in the parent antibiotic and a study of molecular models indicates that this is probably due to steric crowding.

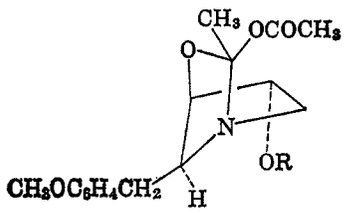
When the nitrogen atom of anisomycin is protonated it is ideally situated to form a hydrogen bond with the acetate carbonyl and this promotes solvolysis of the ester group. This probably explains the rapid hydrolysis of anisomycin observed during its isolation from fermentation liquors.[2] The ester of anisomycin is readily hydrolyzed at pH 8.0.



Acylation of the nitrogen atom of anisomycin renders the lone pair of electrons unavailable for bonding with the acetate carbonyl. Under these circumstances the pyrroldine ring assumes a different conformation with the C-3 and C-4 substituents equatorial and the C-2 substituent is in an axial position. The nmr spectrum of N-benzyloxycarbonyl derivative of anisomycin (**4**, Table I) shows that this is so; the signal for the hydrogen e at C-3 is split into a quadruplet by the adjacent hydrogens at C-2 and C-4 (J = 6.0 and 5.0 cps, respectively), and the hydrogen e at C-4 gives rise to a multiplet at 4.04 ppm.

The proximity of the acetyl group and the nitrogen atom in anisomycin is ideal for the formation of *o*-acetates. Electrophilic attack by CH_3CO^+ at the acetate carbonyl which is already made more polarizable by the nearness of the nitrogen atom will bring about a complete transfer of electrons as indicated.



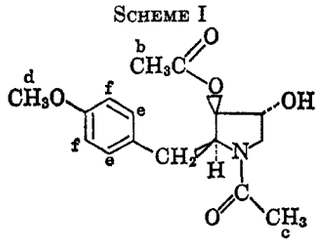


6, R = H
9, R = $COCH_3$

We attempted to prepare such *o*-acetates by reaction of anisomycin with acetic anhydride, but the only product was a highly crystalline substance whose infrared spectrum and chemical properties were in accord with the simple N-acetylanisomycin **5**.[2] However, the nmr spectrum of this material showed three singlets at 1.51, 2.06, and 2.11 ppm which were assigned to C—CH_3, O—CO—CH_3, and N—CO—CH_3, respectively. Integration of this spectrum indicated the ratio 1:2:1 for the relative intensities of the three singlets, which suggests a mixture composed of equal parts of the N-acetyl drivative **5**, and the *o*-acetate **6**. We were unable to separate this mixture into its components.

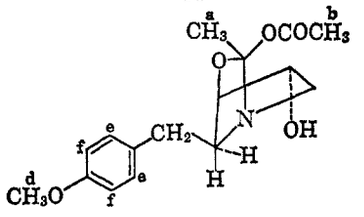
Treatment of anisomycin with acetic anhydride and pyridine under vigorous conditions gave a "triacetate."[2] The nmr of this substance shows three singlets at δ 1.52, 2.05, and 2.11 ppm which are assigned to C—CH_3, —O—COCH_3, and N—CO—CH_3, respectively. The integration for this spectrum gave a total of nine hydrogen atoms for the three methyl signals, but interpretation of an offset spectrum indicated the ratio 1:4:1 for the relative intensities of the three singlets, which is in accord with a mixture of equal parts of the triacetate **7**, and the *o*-acetate **9**. It seems, therefore that acetylation of anisomycin does produce derivatives of the *o*-

SCHEME I



5

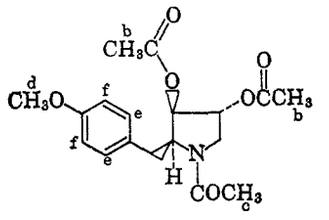
a 1.61 ppm
b 2.06 ppm
c 2.11 ppm



6

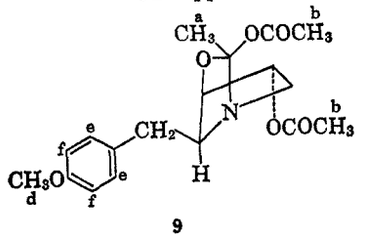
d 3.79 ppm
e 6.83 ppm
f 7.17 ppm

SCHEME II



7

a 1.52 ppm
b 2.05 ppm
c 2.11 ppm

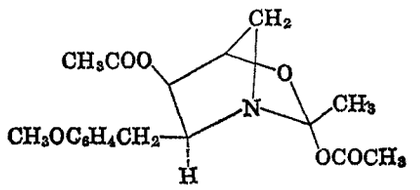


9

d 3.68 ppm
e 6.85 ppm
f 7.17 ppm

acetate, but they are formed together with the normal N-acetyl compounds.

Structure **6**, proposed for the *o*-acetate prepared in the presence of acetic anhydride, is reasonable from a mechanistic point of view, but the *o*-acetate obtained under more vigorous reaction conditions may not necessarily have the analogous structure **9**. We must consider the possibility that the triacetate was derived from an intermediate diacetate (**13**) which can undergo transannular ring closure to produce the *o*-acetates **9** or **10** depending upon which of the two

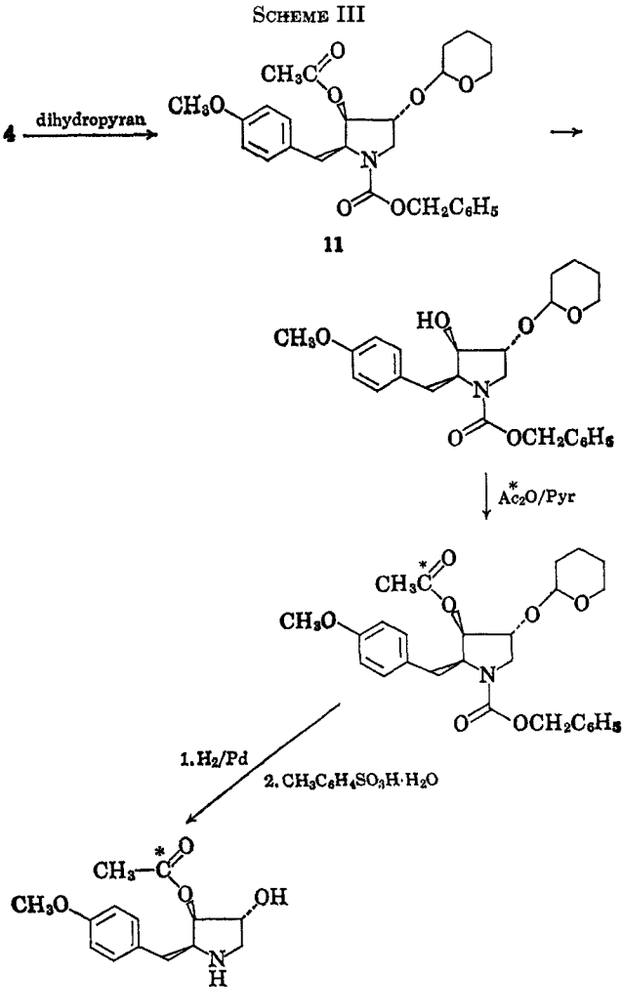


10

acetate functions is involved in the transannular interaction. In order to resolve this problem and to shed light on the *o*-acetate formation, a series of experiments were carried out using samples of anisomycin and isoanisomycin with labeled acetate groups.

Labeled isoanisomycin is readily obtained by treatment of the expoxide **8** with 1-^{14}C-acetate. Samples of 1-^{14}C-acetate labeled anisomycin had been obtained previously by biosynthetic methods,[1] but the degree of incorporation of the radioactive label was too low to be useful in these studies. It was necessary to prepare first the N-benzyloxycarbonyltetrahydropyranyl derivative **11**, and then selectively hydrolyze the acetate ester and reesterify with 1-^{14}C-acetic anhydride in order to obtain sufficiently high levels of radioactivity. Removal of the blocking groups was effected by catalytic hydrogenation followed by crystallization of the anisomycin as its *p*-toluenesulfonate salt (Scheme III).[5]

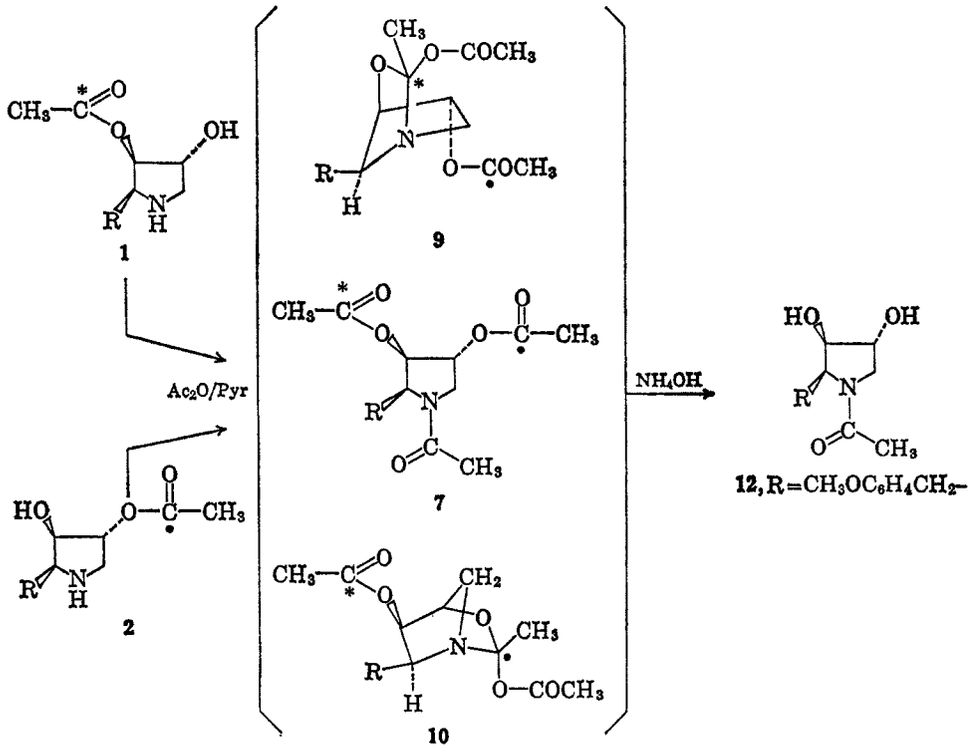
SCHEME III



Labeled samples of anisomycin and isoanisomycin were acetylated using nonradioactive acetic anhydride in pyridine. The products of the two reactions were identical; nmr spectra showed the presence of the two reactions were identical; nmr spectra showed the presence of the N-acetyl derivative (**7**) and an *o*-acetate **9** and/or **10** (Scheme IV).

(5) The author is indebted to Dr. J. J. Beereboom of these laboratories, who first explored the sequence of reactions used for the preparation of 1′-^{14}C-acetate-labeled anisomycin.

SCHEME IV

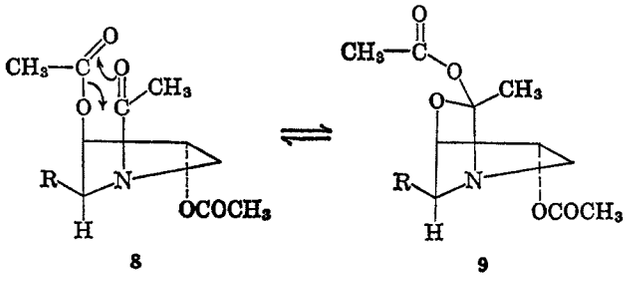


The distribution of radioactive carbon in the "triacetate" is different for the anisomycin and isoanisomycin experiments. If the labeled acetate (asterisk) of anisomycin is involved in *o*-acetate formation, it becomes incorporated into the ring of the bicyclic system **9**, but not in **10**. It follows that the label in isoanisomycin (dot) becomes attached to the nitrogen atom in **10** but not in **9**. Hydrolysis of the "triacetate" mixture by aqueous ammonia causes rapid cleavage of the *o*-acetates and the normal acetate esters to yield N-acetyldeacetylanisomycin **12**. The same product (**12**) should be obtained from **7**, **9**, and **10**, but for the *o*-acetates this results in any oxygen to nitrogen migration of the acetyl group which was involved in formation of the bicyclic system. Thus, if the ester of anisomycin (asterisk) is involved in formation of an *o*-acetate (**9**), the hydrolysis product (**12**) should be radioactive. The two other possible acetylation products from anisomycin (**7** and **10**, asterisk) would give rise to unlabeled N-acetyldeacetylanisomycin (**12**). The same argument applies to the radioactive label (dot) of isoanisomycin which will only appear in the hydrolysis product (**12**) if the bicyclic system **10** were formed in the acetylation reaction. Liquid scintillation counts demonstrated that 27–30% of 1-^{14}C-acetate of anisomycin became incorporated into the N-acetyldeacetylanisomycin, but only 7% of the labeled acetate of isoanisomycin was found in **12**.

Similar experiments which involved the acetylation of unlabeled anisomycin by 1-^{14}C-acetic anhydride in pyridine were not very satisfactory because much ester exchange occurred and the label became more uniformly distributed about the molecule.

The experiments with labeled materials have confirmed the formation of bicyclic *o*-acetate from anisomycin and further shows that **9** is preferable to **10**. This is in complete agreement with our understanding of the mechanism of this reaction and reflects the different extents to which the ester functions of anisomycin and isoanisomycin interact with the nitrogen atom of the pyrrolidine ring. The different pK value for these two compounds is due to the same phenomena.

We were unable to separate the *o*-acetate **9** from the triacetate **7** by conventional methods, and so to prepare the authentic triacetate (**7**) we attempted acetylation of a labeled sample of N-acetyldeacetylanisomycin **12**. We were surprised to find that the triacetate so obtained showed C-methyl, N-acetyl, and O-acetyl signals in the nmr spectrum indicating that the product was again a mixture of **7**, **9**, and **10**. Integrations indicated that about one-half of the mixture was the *o*-acetate; in fact, the spectrum was identical with that of the "triacetate" mixture obtained from anisomycin or isoanisomycin. Hydrolysis regenerated N-acetyldeacetylanisomycin (**12**) which contained the same amount of radioactivity as the samples of starting material. Furthermore, we observed that the ratio of the C-methyl to acetate methyl for the "triacetate" as measured by integration of offset proton magnetic resonance spectra varies with the solvent used for the determination of spectra indicating that the triacetate **7** is in equilibrium with the bicyclic *o*-acetate **9** and possibly with **10**. Equilibrium seems to be rapid at room temperature, and probably involves a four-center reaction of the kind



shown. More evidence was obtained by thin layer chromatography of the "triacetate" mixture, using a benzene–diethylamine–formamide solvent system to develop the chromatogram. Two distinct fractions were observed, but when each fraction was eluted from the plates for determination of pmr spectra, we found that they had reverted to the original mixture of **7** and **9**. Reexamination of the two individual fractions from the first chromatogram showed that each contained the same two distinct zones on the chromatographic plates.

These novel transformations raise interesting questions concerning the conformational properties of highly substituted five-membered rings. It is generally agreed that five-membered rings are not planar, but the pyrrolidine ring has to be severely buckled in order for the transformation **7** → **9** to occur, and the transition state for this reaction requires that two of the three-substituent groups assume a *quasi*-axial configuration. Further studies of this phenomenon are in progress.

Experimental Section

Preparations and physical properties of the following compounds were described in a previous communication:[2] anisomycin (2-*p*-methoxyphenylmethyl-3-acetoxy-4-hydroxypyrrolidine (**1**), deacetylanisomycin (2-*p*-methoxyphenylmethyl-3,4-dihydroxypyrrolidine (**3**), N-acetylanisomycin (2-*p*-methoxyphenylmethyl-3-acetoxy-1-acetyl-4-hydroxypyrrolidine (**5**), N,O-diacetylanisomycin (2-*p*-methoxyphenylmethyl-3,4-diacetoxy-1-acetylpyrrolidine (**7**), 2-*p*-methoxyphenylmethylpyrrolidine 3,4-epoxide (**8**), isoanisomycin (2-*p*-methoxyphenylmethyl-3-*trans*-hydroxy-4-acetoxypyrrolidine (**2**), and N-acetyldeacetylanisomycin (2-*p*-methoxyphenylmethyl-3,4-dihydroxy-1-acetylpyrrolidine (**11**).

2-*p*-Methoxyphenylmethyl-3-acetoxy-4-hydroxy-1-benzylcarbamylpyrrolidine (**4**).—Anisomycin (30 g) was dissolved in a mixture of chloroform (250 ml) and triethylamine (36 ml) at 0°. Benzyl chloroformate (25 ml) was slowly added to the solution with stirring, and the mixture was allowed to warm to room temperature, The chloroform solution was washed with dilute HCl and then with saturated K_2HCO_3 and evaporated to a colorless oil which was crystallized from ether–hexane mixtures (21 g, 46%): mp 69–71°, R_f (tlc) 0.2 (7:3, hexane–ethyl acetate).

Anal. Calcd for $C_{22}H_{25}NO_6$: C, 66.15; H, 6.31; N, 3.51. Found: C, 66.31; H, 6.26; N, 3.55.

Preparation of ^{14}C-Labeled Anisomycin (2-*p*-Methoxyphenylmethyl-3-1′-^{14}C-acetoxy-4-hydroxypyrrolidine).—The benzylcarbamyl derivative **4** (5.0 g) was dissolved in freshly distilled dihydropyran (50 ml) and concentrated HCl (0.5 ml) was added to the solution, which was then stirred at room temperature for 24 hr. Ether (250 ml) was added and the mixture was twice extracted with 2 *N* sodium hydroxide solution. Evaporation of the solvent gave the product as a pale yellow gum which could not be crystallized, R_f (tlc) 0.43 (7:3, hexane–ethyl acetate). The product was not characterized but from a study of its chemical reactions, it was deduced to be *2-p-methoxyphenylmethyl-3-acetoxy-H-2′-tetrahydropyranyloxy-1-benzylcarbamylpyrrolidine* (**11**).

Treatment of the tetrapyranyl derivative (**11**) with dilute HCl in ethanol gave a quantitative yield of the starting benzylcarbamyl compound **4**.

The tetrahydropyramyl derivative was treated with a 1 *N* solution of KOH in ethanol at room temperature for 24 hr. Solvent was removed by evaporation under reduced pressure and the residue was dissolved in water, extracted with ethyl acetate which was then dried with Na_2SO_4 and evaporated to a gum, R_f (tlc) 0.05 (7:3, hexane–ethyl acetate), which lacked ester carbonyl absorption in the infrared spectrum. This substance could not be crystallized, but was reesterified by reaction with 1-^{14}C-acetic anhydride in pyridine at room temperature for 16 hr. Evaporation of the solvent provided a radioactive ester, identical in all respects with 2-*p*-methoxyphenylmethyl-3-acetoxy-H-2′-tetrahydropyranyloxy-1-benzylcarbamylpyrrolidine (**11**) previously prepared from the benzylcarbamyl derivative **4**.

The radioactive ester was dissolved in ethanol (100 ml) and hydrogenated (100 mg of 10% Pd–C; H_2, 50 psi) for 18 hr. *p*-Toluenesulfonic acid monohydrate (540 mg) was added to the filtered mixture which was warmed to provide a clear solution and then evaporated to dryness. A solution of the residue in chloroform was washed with water made basic by addition of Na_2CO_3 solution to pH 10, and the chloroform was dried (Na_2SO_4) and evaporated to yield 1-^{14}C-acetate-labeled anisomycin (mp 145–146° from ethyl acetate), 850 mg (40% yield over-all) identical in every respect with the natural material. Liquid scintillation counts showed the product to contain 7.85–7.91 μc/mmol.

Reaction of ^{14}C-Labeled Anisomycin with Acetic Anhydride.—Radioactive anisomycin (85 mg) labeled in the ester function (7.9 μc/mmol) was converted into N,O-diacetylanisomycin (**7**) by the method previously described. Nmr spectra showed the product to be identical with an authentic sample. The N,O-diacetyl derivative was dissolved in methanol containing 10% of saturated aqueous ammonia, the mixture was stirred at 50° for 1 hr and evaporated to provide N-acetyldeacetylanisomycin (**12**), mp 143–145°.[1] Liquid scintillation counts showed this material to contain 2.36 μc/mmol indicating a 30% transfer of the ^{14}C-acetate from **1** to the nitrogen of **12**.

A repeat of this experiment gave radioactive N-acetyldeacetylanisomycin containing 2.2 μc/mmol (27% retention of radioactivity).

Reaction of 1′-^{14}C-Acetate-labeled Isoanisomycin with Acetic Anhydride.—Radioactive isoanisomycin (2-*p*-methoxyphenylmethyl-3-*trans*-hydroxy-4-1′,^{14}C-acetoxypyrrolidine (**2**) was obtained from the epoxide **8**, and labeled acetic acid.[1] Liquid scintillation counts showed it to contain 11.1 μc/mmol.

This isoanisomycin (100 mg) was subjected to the same treatment described above for anisomycin. The N-acetyldeacetylanisomycin (65 mg) obtained after hydrolysis of the acetylation product contained 0.76 μc/mmol (7% retention of radioactivity).

Reaction of Labeled N-Acetyldeacetylanisomycin with Acetic Anhydride.—2-*p*-Methoxyphenyl-3,4-dihydroxy-1-1′-^{14}C-acetylpyrrolidine (100 mg, 13.7 μc/mmol was dissolved in pyridine (2 ml) and acetic anhydride (0.5 ml). After 10 hr the mixture was worked up by the described procedure to yield N,O-diacetylanisomycin (80 mg, 61%), mp 85–88° (13.6 μc/mmol) identical in all respects with authentic material. Hydrolysis of the product by aqueous ammonia in methanol gave N-acetyldeacetylanisomycin (50 mg, 82%), mp 143–144° (13.75 μc/mmol)

Registry No.—**1**, 2322-08-9; **2**, 15815-58-4; **4**, 15815-59-5; ^{14}C-labeled anicomycin, 16165-20-1; N,O-diacetylanisomycin, 15815-61-9; N-acetyl-deacetylanisomycin, 15815-62-0.

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Partial Asymmetric Synthesis in the Simmons–Smith Reaction. II[1]

S. SAWADA, J. ODA, AND Y. INOUYE

Institute for Chemical Research, Kyoto University, Uji, Kyoto, Japan

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The accelerating and directing influence of the hydroxyl group on the steric course of addition of the Simmons–Smith reagent has been pointed out in some

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