identical on all respects (infrared and nmr spectra) with the material prepared from natural sources.

Registry No.—**3**, 15303-46-5; **4a**, 5909-78-4; **4b**, 5909-84-2; **4c**, 5909-85-3; **5a**, 15303-50-1; **5b**, 5909-79-5; **5c**, 5909-83-1; **6**, 5909-80-8; **9**, 15303-54-5; (±)-α-bourbonene, 13828-07-4.

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Structure of Anisomycin

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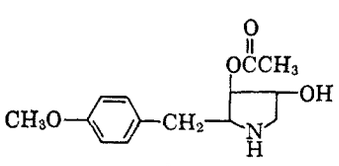
A three-dimensional X-ray diffraction analysis of N-acetylbromoanisomycin has been carried out and the results have been used to determine the structure of anisomycin. The crystals are monoclinic, space group $P2_1$, with a = 11.25 A, b = 7.16 A, c = 11.44 A, β = 112.8°, z = 2. The molecule is a substituted pyrrolidine in which the *p*-methoxy-*m*-bromobenzyl moiety on the 2 position is *cis* to the 3-acetate group which is, in turn, *trans* to a 4-hydroxy function. An analysis of stereoelectronic factors governing ring-opening reactions of cyclopentane derivatives is presented.

The antibiotic anisomycin (**1**) is a fermentation product of various species of *Streptomyces* and has been shown to have widespread activity against certain pathogenic protozoa.[3,4] It has been found to be effective in the treatment of amoebic dysentery and has been used for that purpose.[5] In a recent study of the ipecac alkaloids, Grollman[6] has found that these alkaloids block the aminoacyl–sRNA transfer reaction in protein biosynthesis and that anisomycin effects a similar inhibition.[7] On the basis of these results and certain common structural features between the ipecac alkaloids and anisomycin, Grollman has formulated a structural basis for the inhibition of protein synthesis.

Early chemical studies indicated that **1** has a formula corresponding to $C_{14}H_{19}NO_4$ and possesses a methoxyl group, an acetyl group, and two active hydrogens.[3,8] The basicity of **1** (pK_a 7.75) indicates that the compound is an amine, and subsequent degradation confirmed the presence of a pyrrolidine ring.[8] Further studies established that the gross structural features of anisomycin are best accommodated by the formula of compound **1**.



1

To establish the relative stereochemical relationships of the substituents on **1**, a complex series of transformations of the hydroxyl and acetate groups was undertaken (*vide infra*). As a result of these studies, the groups on the pyrrolidine ring were assigned the all-*trans* configuration. More recent studies[9] have, however, led to results which are difficult to rationalize in terms of this configuration for **1**. As a result, we have determined the crystal structure of a derivative of **1**, N-acetylbromoanisomycin (**2**), to clarify any ambiguity which may exist in the stereochemical assignments.

Experimental Section

N-Acetylbromoanisomycin, $C_{16}H_{20}NO_5Br$ (386.2), is a monoclinic crystal with a = 11.254 ± 0.017, b = 7.160 ± 0.013, c = 11.439 ± 0.018 A, β = 112.8 ± 0.2°; U = 849.7 A³, D_m = 1.501, D_c = 1.506, Z = 2, $F(000)$ = 396; space group $P2_1$ (C^2_2, No. 4); single crystal oscillation and Weissenberg photographs (λ = 1.5418 A).

The crystals (mp 177°) were grown from ethyl acetate as colorless needles elongated along [*b*]. Equi-inclination Weissenberg photographs of the h0l through h4l levels were taken with Cu K radiation, and the relative intensities were obtained by visual estimation against a calibrated strip. Reflections which were too weak to observe were included at one half the local minimum observable value. A total of 1000 independent reflections were recorded.

The structure was solved by the heavy-atom technique. The coordinates of the bromine atom were found from a three-dimensional Patterson synthesis, and the positions of the lighter atoms were obtained from successive two- and three-dimensional Fourier syntheses. Refinement was carried out by differential syntheses until R had dropped to a final value of 10.5%. Hydrogen atoms were ignored throughout. All calculations were carried out on an IBM 7072 computer with programs written in Professor G. A. Jeffrey's laboratory at the University of Pittsburgh.

Results

The final atomic coordinates, with standard deviations given as units in the last place, are shown in Table I. The anisotropic thermal factors of the form

$$B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{23}klb^*c^* + 2B_{13}hla^*c^*$$

are given in Table II. Table III shows an analysis of the agreement between observed and calculated structure factors in terms of the layer index k and of the magnitude of F_0. Bond lengths and angles are given in Table IV. The molecule as it appears in projection

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(3) B. A. Sobin and F. W. Tanner, Jr., *J. Am. Chem. Soc.*, **76**, 4053 (1954).

(4) J. E. Lynch, A. R. English, H. Bauck, and H. Deligianis, *Antibiot. Chemotherapy*, **4**, 844 (1954).

(5) A. B. Cue and J. G. H. Diaz, *Rev. Invest. Bol. Univ. Guadalajara*, **1**, 94 (1961); G. G. Miranda and E. Urbina, *ibid.*, **1**, 95 (1961); J. A. Portilla, *ibid.*, **1**, 95 (1961); A. A. Plata, H. B. Zapata, and V. A. Munoz, *ibid.*, **1**, 96 (1961).

(6) A. P. Grollman, *Proc. Natl. Acad. Sci. U. S.*, **56**, 1867 (1966).

(7) A. P. Grollman, Abstracts, 153rd National Meeting of the American Chemical Society, Miami, Fla., April 1967, 3M.

(8) J. J. Beereboom, K. Butler, F. C. Pennington, and I. A. Solomons, *J. Org. Chem.*, **30**, 2334 (1965).

(9) K. Butler, private communication.

TABLE I

FINAL ATOMIC COORDINATES

Atom	X	Y	Z
Br	0.5236	0.0000	0.8094
O_1	0.3993	0.3616	0.7904
O_2	0.1426	−0.1724	0.3501
O_3	−0.0139	0.0378	0.3292
O_4	0.0964	−0.4651	0.1415
O_5	0.1963	0.2921	0.0172
N	0.1936	−0.0092	0.0691
C_1	0.4293	0.1280	0.6604
C_2	0.3757	0.2932	0.6731
C_3	0.2991	0.3927	0.5511
C_4	0.2810	0.3085	0.4395
C_5	0.3321	0.1365	0.4265
C_6	0.4067	0.0524	0.5371
C_7	0.3407	0.5413	0.7982
C_8	0.3229	0.0636	0.2981
C_9	0.1890	0.0310	0.1957
C_{10}	0.1225	−0.1391	0.2171
C_{11}	0.1782	−0.2947	0.1619
C_{12}	0.1798	−0.2211	0.0455
C_{13}	0.0652	−0.0716	0.3928
C_{14}	0.1013	−0.1219	0.5383
C_{15}	0.1990	0.1221	−0.0103
C_{16}	0.1984	0.0548	−0.0691

TABLE II

FINAL ANISOTROPIC THERMAL FACTORS, A^2

Atom	B_{11}	B_{22}	B_{33}	B_{12}	B_{23}	B_{13}
Br	5.322	4.837	3.976	0.186	−0.845	1.077
O_1	6.723	1.507	4.125	1.829	0.240	2.075
O_2	4.402	1.438	2.636	−0.249	−0.220	2.081
O_3	7.182	6.077	4.885	−1.963	−0.011	2.915
O_4	7.999	6.044	6.595	0.492	0.517	3.370
O_5	6.802	2.905	3.486	−0.096	−0.312	2.938
N	5.942	5.361	5.806	−0.986	−0.512	2.153
C_1	7.898	6.191	5.004	1.200	0.728	1.336
C_2	4.681	2.994	1.005	0.347	−0.023	0.671
C_3	9.843	3.619	5.867	0.930	0.136	3.154
C_4	2.566	3.345	2.273	0.654	0.618	−0.277
C_5	5.558	2.876	5.335	−2.027	1.670	2.215
C_6	4.772	2.586	3.155	−0.959	−0.623	0.528
C_7	12.840	2.309	6.643	1.020	0.892	3.709
C_8	3.799	11.843	4.311	0.510	0.811	2.135
C_9	5.063	0.902	1.935	0.120	0.558	2.861
C_{10}	7.994	3.170	4.013	−0.724	−1.143	3.782
C_{11}	7.918	6.186	4.394	0.520	0.652	2.949
C_{12}	7.760	2.311	4.810	0.754	2.634	3.720
C_{13}	5.602	1.559	4.178	1.249	0.384	2.185
C_{14}	7.737	9.451	3.731	2.510	1.295	2.901
C_{15}	5.921	1.193	3.864	1.029	−0.423	1.540
C_{16}	9.794	14.869	4.622	−0.801	−0.264	5.032

down [*b*] and the labeling of the atoms are shown in Figure 1.

All nonbonded intramolecular contacts less then 4 A were calculated. There are 35 such distances. The shortest, 2.75 A, corresponds to a hydrogen bond between the hydroxyl group (O_5) and the oxygen atom of the N-acetyl group (O_4) linking the molecules into infinite chains. All other contacts are greater than 3.35 A.

A number of least-square planes were calculated in terms of orthogonal axes X, Y, and Z' in order to define more closely the molecular geometry. The plane through C_9, C_{12}, and C_{15}

$$0.9101X + 0.0743Y + 0.4078Z' = -2.2369$$

TABLE III

COMPARISON OF OBSERVED AND CALCULATED STRUCTURE FACTORS AS A FUNCTION OF k AND OF THE MAGNITUDE OF F_0

k	$\Sigma\|F_0\|$	$\Sigma\|F_c\|$	$\Sigma\Delta$	N^a	R
0	3723.3	3751.6	501.3	216	0.135
1	4078.4	4063.5	355.3	253	0.087
2	3325.3	3363.7	337.8	215	0.101
3	2408.4	2427.2	249.5	154	0.104
4	2006.3	2034.6	198.8	162	0.099
All	15541.7	15640.6	1642.7	1000	0.105
Range	$\Sigma\|F_0\|$	$\Sigma\|F_c\|$	$\Sigma\Delta$	N	R
2.4–4.8	581.1	638.6	225.8	165	0.389
4.8–7.2	641.3	686.0	109.8	105	0.171
7.2–9.6	1160.8	1113.4	170.3	138	0.147
9.6–12.0	1198.2	1132.9	144.4	111	0.121
12.0–14.4	1029.5	1004.7	112.5	78	0.109
14.4–16.8	1069.3	1036.8	97.6	69	0.091
16.8–19.2	986.6	974.0	79.5	55	0.081
19.2–21.6	1078.4	1057.6	98.9	53	0.092
21.6–24.0	774.0	789.2	75.0	34	0.097
24.0–26.4	788.6	771.8	50.5	31	0.064
26.4–28.8	825.8	808.8	55.7	30	0.067
28.8–31.2	873.5	878.1	59.1	29	0.068
31.2–33.6	358.3	363.9	27.2	11	0.076
33.6–36.0	596.3	620.3	43.2	17	0.072
36.0–38.4	295.4	306.7	20.2	8	0.068
38.4–40.8	593.4	622.6	47.3	15	0.080
40.8–43.2	508.5	527.5	38.1	12	0.075
43.2–45.6	395.1	392.0	17.9	9	0.045
45.6–50.4	482.9	515.4	48.3	10	0.100
50.4–60.0	505.6	570.7	72.6	9	0.144
60.0–67.2	443.9	471.0	38.2	7	0.086
67.2–120.0	355.2	358.6	10.6	4	0.030

[a] N is the number of reflections included in the group.

TABLE IV

BOND ANGLES[a] AND BOND LENGTHS (A)

C_1–Br	1.86 (2)	C_9–N	1.50 (3)	C_1–C_6	1.44 (3)
C_2–O_1	1.36 (2)	C_{12}–N	1.54 (3)	C_5–C_8	1.52 (3)
C_7–O_1	1.46 (3)	C_{15}–N	1.33 (4)	C_8–C_9	1.53 (3)
C_{10}–O_2	1.47 (2)	C_1–C_2	1.36 (3)	C_9–C_{10}	1.50 (3)
C_{13}–O_2	1.36 (2)	C_2–C_3	1.50 (3)	C_{10}–C_{11}	1.53 (3)
C_{13}–O_3	1.20 (2)	C_3–C_4	1.35 (3)	C_{11}–C_{12}	1.44 (3)
C_{11}–O_4	1.49 (3)	C_4–C_5	1.39 (3)	C_{13}–C_{14}	1.59 (3)
C_{15}–O_5	1.26 (3)	C_5–C_6	1.36 (3)	C_{15}–C_{16}	1.54 (3)

BrC_1C_2	117	$C_5C_8C_9$	118	$C_{10}C_{11}O_4$	109
BrC_1C_6	122	$C_8C_9C_{10}$	114	$C_{10}C_{11}C_{12}$	106
$C_2C_1C_6$	121	C_8C_9N	112	$C_{12}C_{11}O_4$	113
$C_1C_2C_3$	115	$C_{10}C_9N$	102	$C_{11}C_{12}N$	104
$C_1C_2O_1$	120	$C_9C_{10}O_2$	115	C_9NC_{12}	109
$C_3C_2O_1$	125	$C_9C_{10}C_{11}$	102	C_9NC_{15}	124
$C_2O_1C_7$	117	$C_{11}C_{10}O_2$	113	$C_{12}NC_{15}$	127
$C_2C_3C_4$	119	$C_{10}O_2C_{13}$	115	$NC_{15}O_5$	120
$C_1C_6C_5$	124	$O_2C_{13}O_3$	124	$NC_{15}C_{16}$	117
$C_4C_5C_6$	115	$O_2C_{13}C_{14}$	108	$C_{16}C_{15}O_5$	123
$C_4C_5C_8$	122	$O_3C_{13}C_{14}$	128	$C_3C_4C_5$	125
$C_6C_5C_8$	122				

[a] All angles have a standard deviation of ~2°.

shows that the three bonds to the nitrogen atom are distributed in a plane, the nitrogen atom being 0.035 A from the plane, a distance which is not significant. The four atoms of the N-acetyl group lie in the plane

$$0.9142X + 0.0082Y + 0.4052Z' = -2.0442$$

and this plane is coplanar with the bonds to the nitrogen atom. The six atoms in the benzene ring lie in the plane

$$0.8849X - 0.4658Y + 0.0010Z' = -0.4430$$

the maximum departure being 0.24 A for C_2. The substituent atoms Br, O_1, and C_8 lie +0.063, +0.020, and −0.151 A, respectively, from this plane. The methoxy group is coplanar with the benzene ring. The four atoms of the acetoxy group are coplanar, but the five-membered ring, rather surprisingly, is far from planar. The four atoms N, C_9, C_{11}, and C_{12} do not depart significantly from the plane

$$0.9131X + 0.0640Y + 0.4026Z' = -2.2113$$

but the atom C_{10} is 0.617 A from this plane.

Discussion

Figure 1 shows a molecule of **2** as viewed in a *b*-axis projection. An examination of the structure reveals

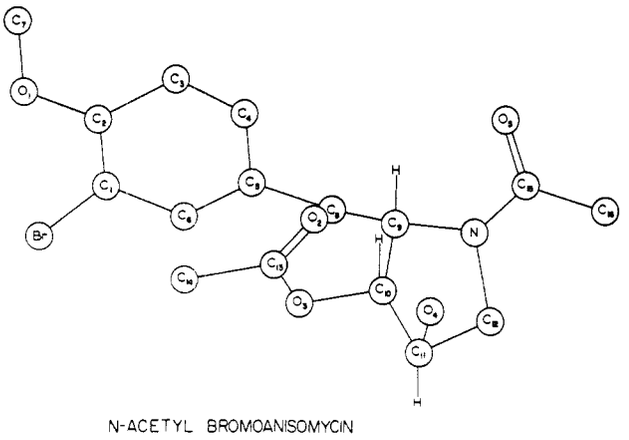


Figure 1

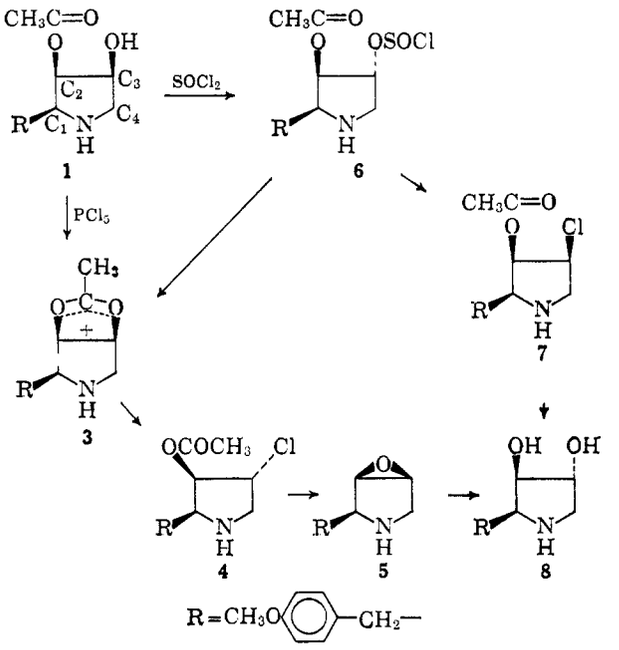
that the relative stereochemical assignment which was previously given to the *p*-methoxybenzyl group is incorrect; this group is *cis* to the adjacent acetate function. As a result, the former interpretation[8] of the reactions which led to the assignment of the all-*trans* configuration to the groups on the pyrrolidine ring must be modified.

Reaction of **1** with phosphorus pentachloride produced compound **4** which contained chlorine and an acetate group. Reaction of **4** with alcoholic potassium hydroxide led to the rapid formation of epoxide **5** (see Scheme I). Therefore, the chlorine atom is *trans* to the acetate group. The stereochemical assignment given to **4** can be deduced using mechanistic principles which have been well developed from comprehensive studies of the reactions of cyclohexane derivatives, but which do not appear to have been applied in the cyclopentane series.

Phosphorus halides are known to react with alcohols by first forming a phosphorus ester. These esters are usually displaced with inversion of configuration at carbon by a halide ion acting as a nucleophile.[10] However, if a neighboring group is present which can act as a nucleophile, it can frequently intercede and replace the halide ion as the nucleophile.[11-13] Since the acetate group is *trans* to the hydroxyl group in anisomycin, participation by acetate will be favored and should lead to ion **3**.[14]

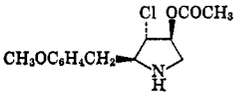
SCHEME I

TRANSFORMATIONS OF ANISOMYCIN



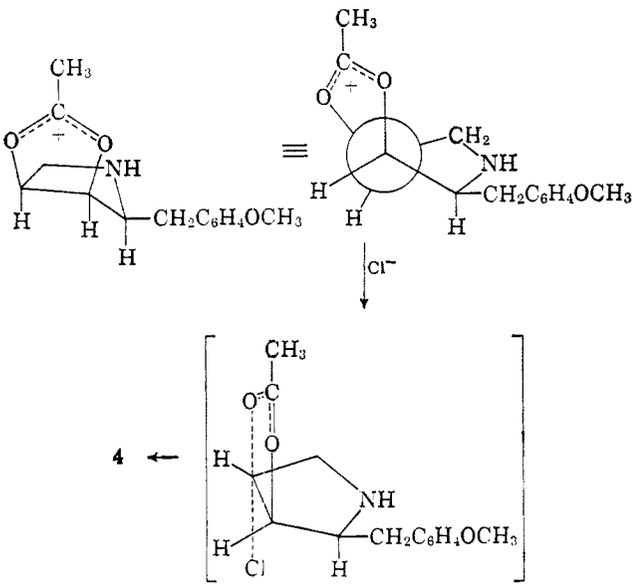
As a result of the extensive studies by Winstein[15] on neighboring-group effects, it is clear that, for participation to be important, the neighboring and leaving groups must be capable of achieving a *trans* orientation. The energy of the transition state for the displacement step will be a minimum when the atoms involved are all coplanar. From the principle of microscopic reversibility, it follows that in ring-opening reactions a similar stereochemical requirement will exist; this has been amply demonstrated in the literature on steroid reactions.[16] If we concede that the benzyl group in **3** prefers to be in an equatorial position and apply the principles outlined above, it becomes immediately apparent that the lowest energy transition state for ring opening would be reached by an attack of the nucleophile on C_3 and not on C_2. This leads to the conclusion that the thermodynamically less stable isomer[17] should be the kinetically favored product, and therefore we have formulated the chloroacetate as **4** and the epoxide as **5**.[18] (See Scheme II.)

(10) H. J. Lucas and C. W. Gould, *J. Am. Chem. Soc.*, **63**, 2541 (1941).
(11) S. Winstein, *ibid.*, **64**, 2761 (1942).
(12) J. P. Schaefer and D. S. Weinberg, *J. Org. Chem.*, **30**, 2635 (1965).
(13) J. P. Schaefer and D. S. Weinberg, *ibid.*, **30**, 2640 (1965).
(14) S. Winstein, H. V. Hess, and R. E. Buckles, *J. Am. Chem. Soc.*, **64**, 2796 (1942).
(15) S. Winstein and R. E. Buckles, *ibid.*, **64**, 2780 (1942), *et seq.*
(16) See G. H. Alt and D. H. R. Barton, *J. Chem. Soc.*, 4284 (1954), and references cited.
(17) R. B. Bates, E. J. Eisenbraun, and S. M. McElvain, *J. Am. Chem. Soc.*, **80**, 3413 (1958).
(18) There are situations in which the initially formed product of an addition reaction subsequently rearranges to a more stable product, as, for example, in the bromination of cholesterol.[19] If this occurs in the present instance, the structure of the isolated product would be that shown below which would also give **5** on reaction with alkali.



(19) D. H. R. Barton and E. Miller, *ibid.*, **72**, 1066 (1950).

SCHEME II



Reaction of **1** with thionyl chloride gave a crystalline chlorosulfite, **6**, which decomposed on heating to a mixture of two chloroacetates, **4** and **7**; the composition of this mixture was dependent upon the reaction conditions. Reaction of **7** with alcoholic potassium hydroxide gave a diol, **8**, which was identical with the product obtained on saponification of **1**. Since the conversion of **7** to **8** involves a Walden inversion, **7** must have the all-*cis* configuration.

The remarkable stereoselectivity of the ring-opening reaction described above is also manifested in the reaction of the epoxide with a variety of nucleophiles. When **5** was treated with acetic acid and hydrolyzed, only diol **8** was obtained. Reaction of **5** with seventeen other nucleophiles (alkoxides, amines, and mercaptans) proceeded smoothly. And, in every case, only a single product was isolated in high yield. From this series of experiments, it appears that reactions of epoxides of cyclopentane derivatives are subject to the same stereoelectronic factors which are operative in the cyclohexane series and that these are highly selective.

Registry No.—**1**, 2322-08-9; **2**, 14669-64-8.

Acknowledgment.—We wish to thank Dr. Kenneth Butler for bringing this problem to our attention and for supplying us with crystals of compound **2**.

Peptide Synthesis *via* Oxidation of N-Acyl-α-amino Acid Phenylhydrazides. II. Benzyloxycarbonyl Peptide Phenylhydrazides[1]

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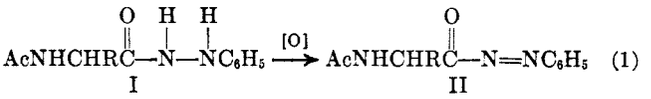
Received July 11, 1967

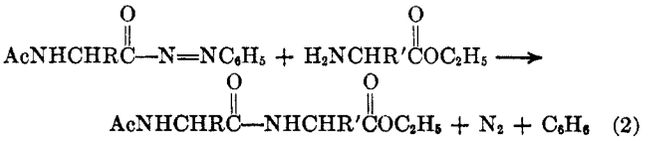
Several benzyloxycarbonyl-α-amino acid phenylhydrazides were oxidized to give crystalline benzyloxycarbonyl-α-amino acid phenyldiimides. These compounds have an n → π* transition at 450 mμ which causes a positive Cotton effect in their optical rotatory dispersion curves and a positive ellipticity in their circular dichroism curves. The benzyloxycarbonylamino acid phenyldiimides reacted with amino acid phenylhydrazides to give benzyloxycarbonyl dipeptide phenylhydrazides in good yield with little or no racemization. The physical properties (infrared, ORD, melting point, and tlc R_f values) of these compounds were the same as those of benzyloxycarbonyl dipeptide phenylhydrazides prepared by a papain-catalyzed reaction between benzyloxycarbonylamino acids and -amino acid phenylhydrazides. The application of this method to higher peptides was shown by the reaction between benzyloxycarbonylglycyl-L-phenylalanine phenyldiimide with glycyl-L-phenylalanine phenylhydrazide to give benzyloxycarbonylglycyl-L-phenylalanylglycyl-L-phenylalanine phenylhydrazide.

It is becoming apparent that the phenylhydrazide group is an effective carboxyl blocking group for amino acids. N-acyl-α-amino acid phenylhydrazides (I) are easily prepared from the acid chloride, the azide or by papain-catalyzed reactions. This group is stable toward mild acid or base. There have been several procedures reported for the removal of the phenylhydrazide group from N-acyl-α-amino acid phenylhydrazides. These all consist of oxidizing the phenylhydrazides in aqueous solutions to give essentially quantitative yields of the acylated amino acids or peptides.[3] We have recently shown that N-acyl-α-amino acid phenylhydrazides may be oxidized in nonaqueous solvents to give N-acyl-α-amino acid phenyldiimides (II).[2]

The usefulness of these N-acyl-α-amino acid phenyldiimides (II) in peptide synthesis was demonstrated by coupling them with amino acid ethyl esters giving N-acyl dipeptide ethyl esters. Several dipeptides were prepared by the method with little racemization. The reactions are formulated in eq 1 and 2.



(1)



(2)

We now wish to report the extension of this method of peptide synthesis to include the reaction of N-acyl-α-amino acid phenyldiimides with α-amino acid phenylhydrazides to give N-acyl dipeptide phenylhydrazides,

(1) Supported in part by U. S. Public Health Service Research Grant G. M. 11835 from the National Institutes of Health. For part I of this series, see ref 2.

(2) H. B. Milne and W. D. Kilday, *J. Org. Chem.*, **30**, 64 (1965).

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