STEREOSELCTIVE SYNTHESIS OF THE GEOMETRIC AND OPTICAL ISOMERS OF UNSATURATED 3-MONO- AND 3,6-DISUBSTITUTED 2,5-PIPERAZINEDIONES

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Abstract — Four kinds of naturally occurring 3,6-dibenzylidene (1)-, 3-(p)-anisilidene-6-benzylidene (2)-, 3-isobutylidene-6-benzylidene (3; antibiotic albonoursin)-, and 3-benzyl-6-benzylidene-2,5-piperazinediones (4), isolated from *Streptomyces* and *Actinomyces* strains, and their possible geometric and optical isomers were stereoselectively synthesized. Based on the spectral data and the independent preparation, the configuration and conformation of the natural products 1, 2, and 3 could be clearly determined to be (3Z, 6Z)-isomers and 4 to be (3R, 6Z)-isomer. Particularly, from the NMR and CD spectral data, the useful criterion for the configurational determination of the geometry of exocyclic C=C bond was obtained and the conformation of the half boat structure of 2,5-piperazinediones was unambiguously confirmed. Moreover, the methylation of 1, 2, and 3 was achieved to give mainly three kinds of derivatives, 1-, 4-mono-, and 1,4-dimethylated 1, 2, and 3 respectively.

1. Introduction

Antibiotic albonoursin (3) was first isolated, together with 3,6-dibenzylidene (1)- and/or 3-(p)-anisilidene-6-benzylidene (2)-, and 3-benzyl-6-benzylidene-2,5-piperazinediones (4) (hereafter, 2,5-piperazinedione is abbreviated as PDO), from the culture filtrate of *Streptomyces albus var. fungatus*, *Streptomyces noursei* and *Streptomyces thioluteus* by Brown and Kelley and by other workers.[1-4] Recently, albonoursin was also isolated from the culture filtrate of *Actinomyces tumemacerans* by Fukushima *et al.*,[5] as summarized in Table 1.

Table 1. Naturally occurring 1, 2, 3, and 4

Natural products	Produced by	Physical constants[a]
1	*Streptomyces noursei*	Mp 298-300 °C[b]
	Streptomyces thioluteus	UV 234 nm (log ε=3.9)
		398 nm (log ε=4.3)
2	*Streptomyces thioluteus*	Mp 270-273 °C[c]
		UV 350 nm
		398 nm
3	*Streptomyces albus var.*	Mp 272 °C[b]
(Albonoursin)	*fungatus*	UV 234 nm (log ε=3.9)
	Streptomyces noursei	318 nm (log ε=4.4)
	Streptomyces thioluteus	
	Actinomyces tumamecerans	
4	*Streptomyces noursei*	Mp 288.5-290 °C[d]
		UV 296 nm (log ε=4.1)
		$[\alpha]_D^{25}$ -520° (c 0.028)[e]

a) Appeared in the literatures. b) Ref. 7. c) Ref. 6. d) Ref. 4 and 8.
e) Recorded in CF_3COOH.

Of late it was reported that the naturally occurring albonoursin (3) exhibited antibacterial activity and inhibited the growth of transplantable solid tumors in mice,[5] though the other natural products 1, 2, and 4 have no

special biological activity.[6] Therefore, much attention has been directed to the available synthetic method for the unsymmetric unsaturated 3-mono- and 3,6-disubstituted PDO, the asymmetric 3-optically active 6-unsaturated PDO and their analogs. In addition, it is very interesting and important to investigate the correlation between the structure and the bioactivity.

As Fig. 1 shows, the chemical structure of albonoursin (3) was confirmed unambiguously to be 3-isobutylidene-6-benzylidene-PDO, independently, by Khokhlov and Lokshin,[7] Brown *et al.*,[8] and Vondracek and Vanek,[4] respectively. Moreover, the chemical structures of 1, 2, and 4 were also confirmed.[6,7,8]

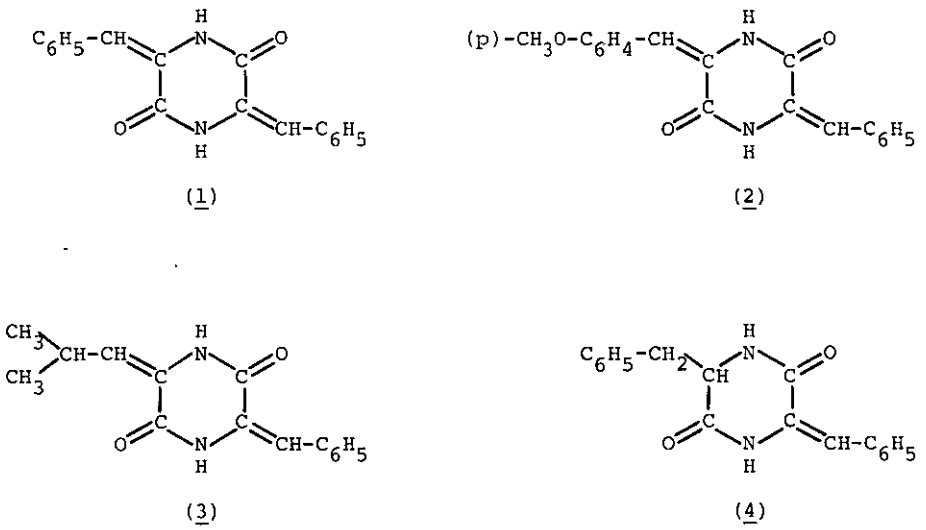
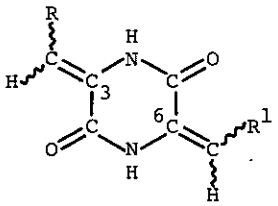


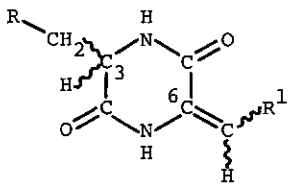
Fig. 1. Chemical structures of four naturally occurring unsaturated 6- and 3,6-disubstituted PDO

The syntheses of 1, 2, and 3 were already accomplished by Sasaki,[9] Gerber,[6] and Shin *et al.*,[10-12] respectively, and the present author further reported the useful and facile synthesis of numerous analogs of 1-3.[12,13] However, no report appeared in the literature on the stereoselective synthetic method for the geometric and optical isomers as well as the configurational and the conformational determinations of all the natural products 1, 2, 3, and 4. All the possible geometric and optical isomers of 1, 2, 3, and 4, attaining fifteen kinds, are presented in Table 2.

Table 2. All the possible geometric and optical isomers of 1-3 and 4



(1, 2, and 3)



(4)

Compound number (No.)	Possible isomer			
1	(3Z, 6Z)-1	(3E, 6Z)-1	(3E, 6E)-1	
2	(3Z, 6Z)-2	(3E, 6Z)-2	(3Z, 6E)-2	(3E, 6E)-2
3	(3Z, 6Z)-3	(3E, 6Z)-3	(3Z, 6E)-3	(3E, 6E)-3
4	(3R, 6Z)-4	(3S, 6Z)-4	(3R, 6E)-4	(3S, 6E)-4

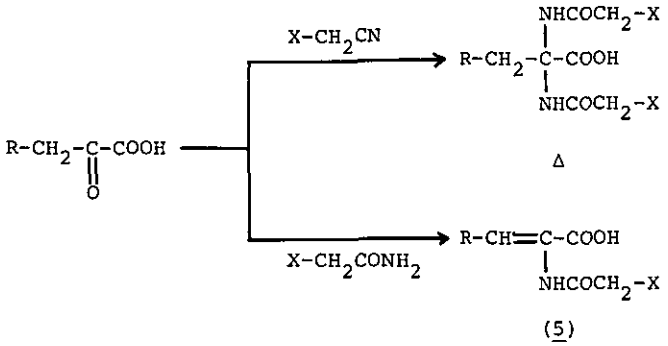
In previous papers,[14-16] the author reported briefly on the synthesis and the configuration of albonoursin (3) and its all the geometric isomers. More recently, the synthesis of the naturally occurring 4 and its all the isomers has also been reported. Because of the pharmacological interests in the relation between the natural products 1-3 and 4 and their stereochemical isomers, and furthermore in the correlation between the structure and the bioactivity, the synthetic methods for all the geometric and the optical isomers of 1-3 and 4 were enthusiastically pursued and successful.[17,18]

As the results, the configurational and the conformational assignments of all the new products thus prepared were established on the basis of infrared (IR), ultraviolet (UV), nuclear magnetic resonance (NMR), and circular dichroism (CD) spectroscopic analyses as well as the satisfactory elemental analysis and the chemical determination by the independent preparation.

2. Preparation of Starting Materials

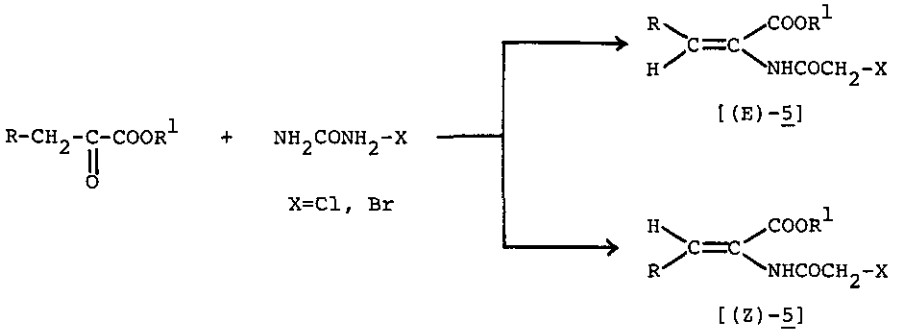
In order to synthesize the desired unsaturated 3-alkylidene- and 3-arylidene-PDO derivatives (6), it is necessary to establish the facile and available synthetic method for 2-phthaliminoacetylamino-[15] and 2-haloacetylamino-2-

alkenoic acid and their esters (5; acyl-α-dehydroamino acids),[13,19] which are thought to be the most useful starting material for the synthesis of 1, 2, and 3. Therefore, various acidic catalysts and dehydrating agents were thoroughly reexamined in the condensation of 2-oxoalkanoic acid and its ester with haloacetnitrile or haloacetamide,[11] aimed at preparing the compound 5. As a result,



(5)

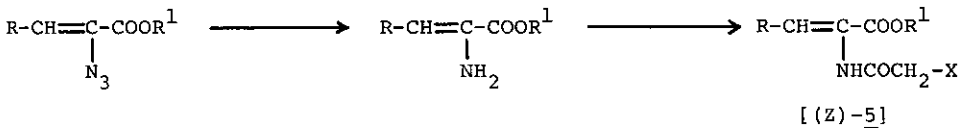
it was found that the condensation reaction of alkyl 2-oxoalkanoate with haloacetamide in benzene in the presence of a mixture of concentrated sulfuric acid and phosphoryl chloride as an acidic catalyst under reflux gave the expected 5 (R=H, CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, and C_6H_5) in a good yield.



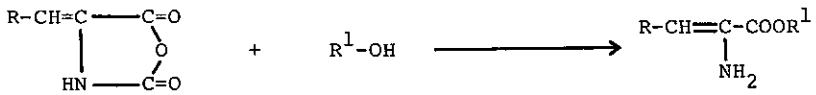
Since the condensation products (5) thus obtained were found to be a mixture of (E)- and (Z)-geometric isomers by a ratio *ca.* 1 : 3.5, evaluated from the intensity of the olefinic proton in the 3-position in the NMR spectrum of 5, the crude syrupy product was subjected to a chromatography for the pure separation of the isomers. Thus, the mixture was separated purely on a silica gel column using benzene or a mixture of benzene-acetone as the eluent to give a colorless

syrup thought to be the (E)-isomer as the first fraction and colorless crystals to be the (Z)-isomer as the second fraction, as methioned hereinafter. Consequently, it seems to be one of the most effective methods for the synthesis of (E)- and (Z)-geometric isomers of 5.

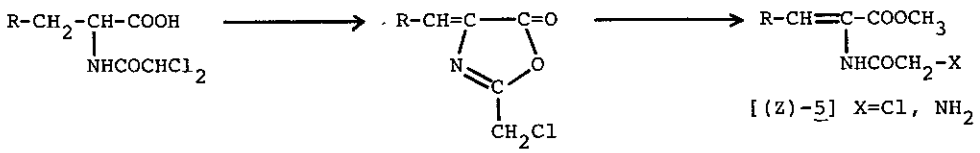
On the other hand, previously, it was reported that the acylation of alkyl (Z)-2-amino-2-alkenoate, obtained by the reduction of alkyl (Z)-2-azido-2-alkenoate[20,21] with aluminum-amalgam,[22,23] with haloacetyl halide or phthalylglycyl chloride gave several (Z)-geometric isomers of 5 (R=CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, and C_6H_5) in fairly good yields. More recently, very useful one-pot



synthesis of alkyl (Z)-2-amino-2-alkenoate was established by the coupling of N-carboxy-α-dehydroamino acid anhydride, newly derived from benzyloxycarbonyl (Cbz)-α-dehydroamino acid, with an appropriate alcohol.[24,25]

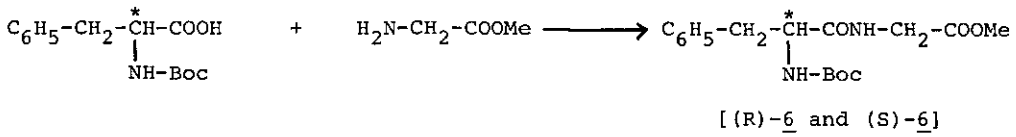


Furthermore, as an another synthetic route of 5, 2-chloromethyl-4-isobutylidene-5-oxazolone, prepared by the reaction of dichloroacetyl-leucine with acetic anhydride, was hydrolyzed to give chloroacetyl-α-dehydroleucine, which was subsequently treated with ammonia in methanol to give glycyl-α-dehydroleucine. The dehydrodipeptide thus obtained was esterified with methanol-hydrogen chloride to give the corresponding methyl ester (5).[10,11]



On the other hand, as the starting materials for the synthesis of (R)-3- and (S)-3-benzyl-PDO, both t-butoxycarbonyl (Boc)-(R)- and (S)-phenylalanyl-

glycine methyl esters (6) were prepared by the coupling of the individual Boc-(R)- and (S)-phenylalanine with glycine methyl ester in CH_2Cl_2, according to the procedure of Nitecki *et al.*[28]



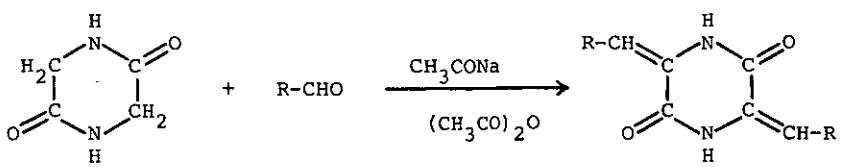
[(R)-6 and (S)-6]

According to the criterion regarding the configuration of exocyclic double bond of PDO reported previously,[26,27] the geometry of 5 could be readily determined. By comparison of the chemical shifts of the NH, olefinic and 3-alkyl (both methylene and methine) protons between (E)- and (Z)-isomers, all the chemical shifts of NH, olefinic, and 3-alkyl protons of 5 in the syrupy state, obtained by the condensation of alkyl 2-oxoalkanoate with amide, resonate at fields lower within 0.5-0.7, 0.2-0.6, and 0.3-0.8 ppm respectively than those of 5 in the crystalline state. Therefore, it was readily confirmed that the former syrup obtained as the first fraction had the (E)-configuration, while the latter crystals as the second fraction had the (Z)-configuration.

From the above results and facts,[26,27] the evidence concerning the NMR spectral data of 5 methioned above can also be used to determine the configuration of the other α,β-unsaturated α-substituted α-amino acid system reported previously. In addition, in the IR spectrum of 5, the characteristic difference between the (E)- and (Z)-geometric isomers could not be recognized.

3. Preparation of 2,5-Piperazinediones (PDO)

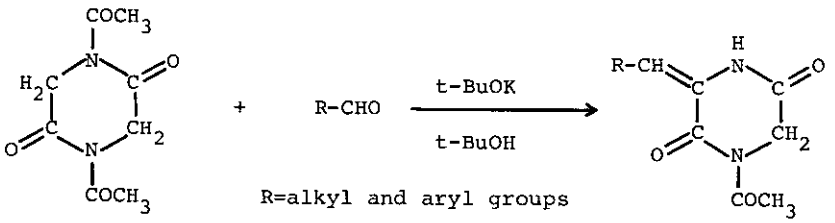
In 1921, Sasaki succeeded first in the synthesis of 3,6-dibenzylidene-PDO by the condensation of glycine anhydride with benzaldehyde in the presence of anhydrous sodium acetate in acetic anhydride at 100 °C.[9] However, this method is restricted to the condensation with alkylaldehyde and to the synthesis of



R=aryl group

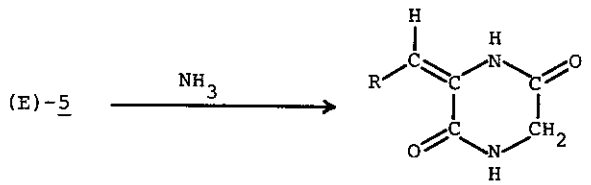
3-arylidene- and 3,6-unsymmetric diarylidene-PDO.

On the other hand, after accomplishing our works on the synthesis of unsymmetric unsaturated 3-mono- and 3,6-disubstituted PDO, Gallina and Liberatori reported the useful synthesis of 3-alkylidene-PDO derivatives by the condensation of 1,4-diacetylglycine anhydride with an appropriate aldehyde in the presence of potassium t-butoxide in t-butanol at 0 °C, followed by the deacetylation of the resulting 1-acetyl-3-alkylidene-PDO (9).[29]

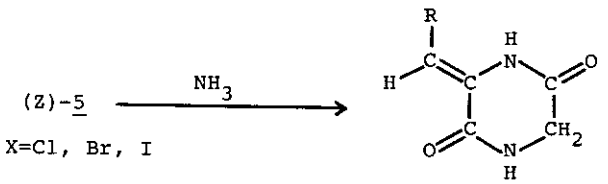


(9)

The individual alkyl (E)- and (Z)-2-haloacetylamino-2-alkenoates [(E)-5 and (Z)-5] were subjected to the cyclization with alcoholic ammonia, according to the method reported previously.[12,16,30] In general, when the compound (E)- or (Z)-5 was treated with an excess methanol or ethanol solution saturated with gaseous ammonia under cooling and then allowed to stand at room temperature for



[(3E)-7]



[(3Z)-7]

a; R=C_6H_5, b; R=(p)-CH_3O-C_6H_4, c; R=i-C_3H_7

a day, the cyclization gradually occurred to deposit amorphous crystals. The crude crystals were recrystallized from boiling acetic acid to give colorless small needles or powder, which were identified as (E)-3- and (Z)-3-alkylidene-PDO [(3E)-7 and (3Z)-7] respectively. From the result, in the case of bromoacetyl derivative of 5, the yield of 7 was found to be comparatively high. Moreover, after the halogen exchange of chlorine or bromine to iodine by the treatment with potassium iodide in acetone, the resulting iodoacetyl derivative was cyclized similarly with ammonia to give 7 in a further good yield.[27] The yield and the melting point of 7 are listed in Table 3.

Table 3. The yields and melting points of 7

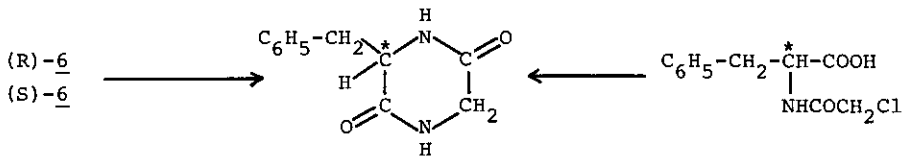
	R	Geometry	Yield (%)	Mp °C (decomp.)
7a	C_6H_5	E	55.0	270-271
		Z	65.0	260-262
7b	(p)-CH_3O-C_6H_4	E	93.0[a]	258-260
		Z	97.3[b]	243-245
	i-C_3H_7	E	47.9	258-259
		Z	57.0	269-271

a) Obtained by the deacetylation of (3E)-9. b) Obtained by the deacetylation of (3Z)-9.

From the NMR spectral data,[27] the compound 7 thus obtained may be summarized as follows: (1) The chemical shifts of NH and 3-methylene protons are lower, but those of olefinic protons are higher than those of 5, reflecting the change in the coplanarity of the molecules. (2) The olefinic and methylene protons of the (Z)-isomer of 7 resonate at lower magnetic field (Δδ 0.45-0.54 and 0.04-0.10 ppm respectively) compared with that of the (E)-isomer, whereas 3-alkyl protons of the (Z)-isomer resonate at a higher magnetic field (Δδ 0.27-0.91 ppm). These facts seem to indicate a flattened half boat-configuration.[31]

On the other hand, according to the procedure of Nitecki *et al.*,[28] the individual (R)-6 and (S)-6 were cyclized to give the expected (R)-3- and (S)-3-benzyl-PDO [(3R)-8 and (3S)-8] respectively in a good yield. These compounds

were also obtained in a fairly good yield, independently by the cyclization of (R)- and (S)-haloacetyl-phenylalanine ethyl ester with excess ammonia.



[(3R)-8 and (3S)-8]

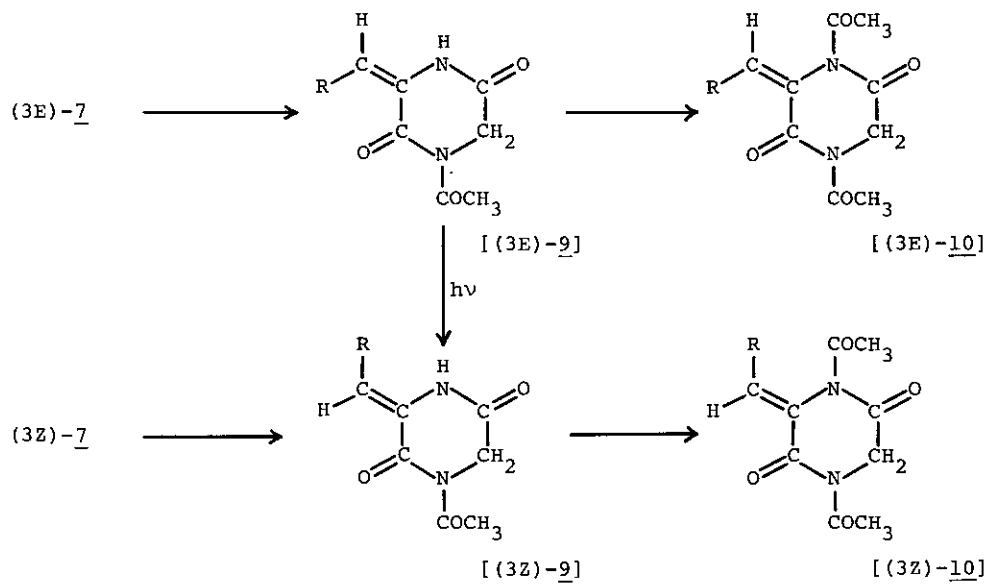
(3R)-8: 92% mp 254-255 °C $[\alpha]_D^{25}$ 106.7° (*c* 0.2)*

(3S)-8: 93% mp 254-255 °C $[\alpha]_D^{25}$ -101.7° (*c* 0.2)*

*Recorded in CF_3COOH

4. Acetylation of PDO with Acetic Anhydride

The acetylation of the 1- and 4-positions of 7 and 8 was thoroughly studied here,[13,14,17] since the condensation reaction of 1-mono- or 1,4-diacetyl-PDO derivatives with an appropriate aldehyde proceeded more smoothly and in higher yield than that of 1,4-free PDO derivative with aldehyde to give the desired unsaturated 3- and 3,6-disubstituted PDO. As a result, the acetylation of the



[(3E)-9] [(3E)-10]

[(3Z)-9] [(3Z)-10]

geometric isomers of 3-benzylidene [(3E)- and (3Z)-7a]- and 3-isobutylidene-PDO [(3E)- and (3Z)-7c] with acetic anhydride under reflux for *ca.* 3 hours gave predominantly the corresponding 1-acetyl-PDO derivatives [(3Z)-9] and 1,4-diacetyl-PDO derivatives [(3E)-10] respectively.[13,14]

This diagnostic chemical behavior to distinguish (E)- and (Z)-alkylidene or arylidene groups will be caused by the steric hindrance of alkyl or aryl group at the 3-position, and this method is applicable even when only one isomer of 7 is in hand. However, it was found that the prolonged acetylation of (3Z)-9 in refluxing acetic anhydride gave a further acetylated derivatives; 1,4-diacetyl-(Z)-3-substituted PDO [(3Z)-10]. Furthermore, 3-(p)-anisilidene-PDO derivatives (7b, 9b, and 10b) were prepared from an another route as follows. According to the procedure reported previously by Porter and Sammes,[32] the photoisomerization of 1-acetyl-(Z)-3-(p)-anisilidene-PDO [(3Z)-9b] gave the corresponding (E)-3-anisilidene derivative [(3E)-9b], but further acetylation of (3E)-9b to 1,4-diacetyl-(E)-3-(p)-anisilidene-PDO [(3E)-10b] failed.

Table 4. The physical constants of 9 and 10

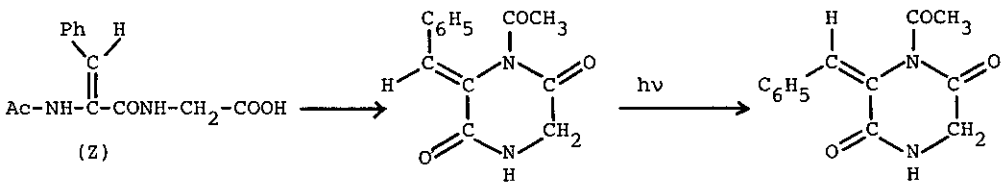
	R	Geometry	Mp °C	NMR spectrum, δ* -CH= (J_{Hz})
9a	C_6H_5	E	157-158	6.58
		Z	201-202	7.15
9b	(p)-CH_3O-C_6H_4	E	173-174	6.50
		Z	178-180	7.12
9c	i-C_3H_7	E	143-145	5.59 (10.0)
		Z	147-148	6.22 (10.0)
10a	C_6H_5	E	126-127	7.13
		Z	149-150	7.50
10b	(p)-CH_3O-C_6H_4	E	not isolated	
		Z	159-160	7.44
10c	i-C_3H_7	E	syrup	6.18 (11.0)
		Z	108-109	6.52 (11.0)

*Measured in $CDCl_3$.

In consequence, the compound (3Z)-9, derived by the acetylation of (3Z)-7, was found to be in complete agreement with the product prepared by the condensation of 1,4-diacetylglycine anhydride with aldehyde by Gallina and Liberatori method.[29] In order to isolate purely the acetylated products, not only the individual crude (E)- and (Z)-isomers but also their mixture was subjected to chromatography on a silica gel column using a mixture of benzene and acetone as the eluent.

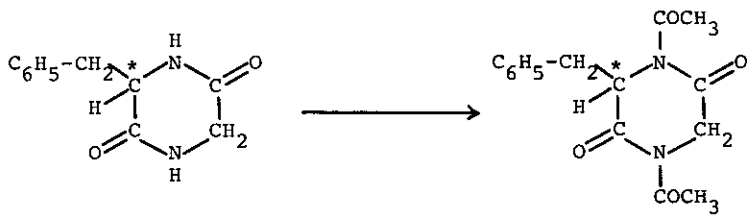
The subsequent deacetylation of 1-acetyl- and 1,4-diacetyl-PDO derivatives [(3E)- and (3Z)-9, 10] with hydrazine hydrate gave pure (3E)-7 and (3Z)-7 respectively in an almost quantitative yield. As a result, except for the preparation of (E)-3-(p)-anisilidene-PDO [(3E)-7b], all the geometric isomers of 7, 9, and 10 were synthesized here, as summarized in Tables 3 and 4.

In particular, as is shown in following figure, the synthesis of 4-acetyl-(E)-3-benzylidene-PDO, which derived by the successive cyclization of N-acetyl-dehydrophenylalanylglycine (Ac-dehydro-Phe-Gly-OH) with acetic anhydride and the photoisomerization of the resulting (3Z)-9, was appeared in the literature.[33]



[(3Z)-9a] [(3E)-9a]

On the other hand, the acetylation of the individual (3R)-8 and (3S)-8 with acetic anhydride by the usual method gave 1,4-diacetyl-(R)-3- and (S)-3-benzyl-PDO [(3R)-11 and (3S)-11] in good yields respectively. In this case, the corresponding monoacetylated derivative was found to be not prepared.



[(3R)- and (3S)-8] [(3R)- and (3S)-11]

(3R)-11: 86% mp 101 °C $[\alpha]_D^{25}$ 79.5° (c 1.0)*

(3S)-11: 90% mp 100.5 °C $[\alpha]_D^{25}$ -82.5° (c 1.0)*

*Recorded in ethanol

The NMR spectral data of 7, 9, and 10 show that the coplanarity of carbonyl function and the exocyclic unsaturated bond in cyclized compounds reflect enhanced deshielding effect of the carbonyl group (Δδ value of olefinic and methine protons between (3E)- and (3Z)-isomers attained 0.34-0.62 and 0.75-1.03 ppm respectively) and that trans protons to the carbonyl function resonate commonly at the upper field.[12,13,14] Similar phenomenon has been observed in 3-alkylidene- and 3-benzylidene-1-hydroxy-PDO in the author's recent works[25,30] and also reported in the case of 3-benzylidene-6-methyl-PDO,[34] γ-butyrolactone,[35] creatine,[36] and further unsaturated 3,6-disubstituted-PDO as methioned in the following.

5. Condensation of Acetyl-PDO with Aldehyde

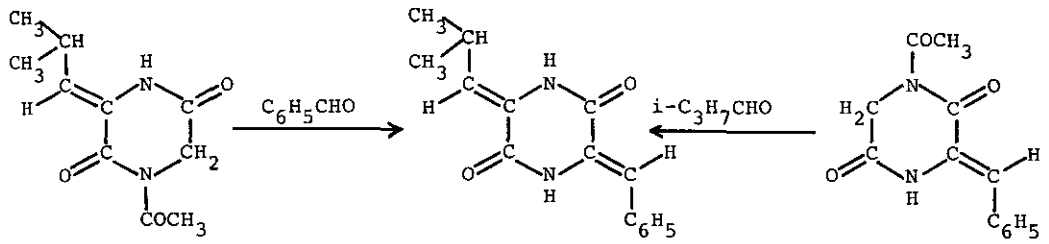
In the condensation reaction of the individual 7, 9, 10, and 11 with an appropriate aldehyde, three typical procedures have been well-known as follows:

1) Procedure A: Condensation of glycine anhydride, saturated or unsaturated 3-substituted PDO with arylaldehyde in acetic anhydride in the presence of anhydrous sodium acetate under reflux.[9]

2) Procedure B: Condensation of the individual 1-acetyl- and 1,4-diacetyl-glycine anhydride or 1-acetyl- and 1,4-diacetyl-3-substituted PDO with arylaldehyde in an appropriate solvent in the presence of base, such as triethylamine, at 100 °C.[37]

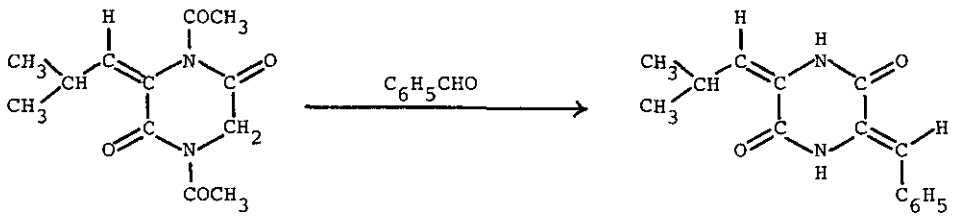
3) Procedure C: Similar work up with alkyl- or arylaldehyde in t-butanol in the presence of potassium t-butoxide at 0 °C.[29]

According to the Procedure B, treatment of 1-acetyl-(Z)-3-isobutylidene-PDO [(3Z)-9c] with benzaldehyde gave crystals, which were found to be in complete agreement with the product prepared by the condensation of 1-acetyl-(Z)-3-benzylidene-PDO with isobutyraldehyde by means of Procedure C. This result indicates clearly that the configurational structure of the products resulted from the above two routes is quite identical with (Z)-3-isobutylidene-(Z)-6-benzylidene-PDO [(3Z, 6Z)-3][13]



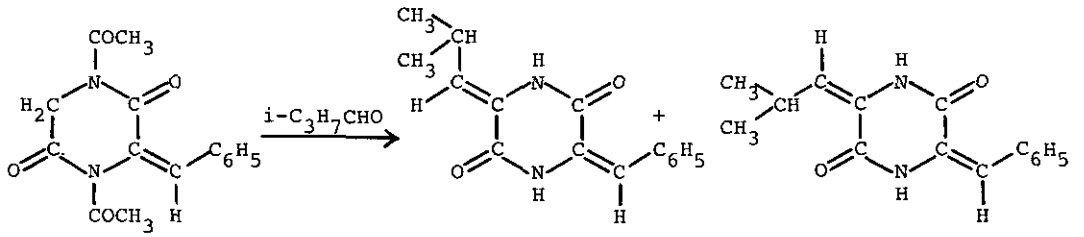
[(3Z)-9c] [(3Z, 6Z)-3] [(3Z)-9a]

The similar condensation of 1,4-diacetyl-(E)-3-isobutylidene [(3E)-10c] with benzaldehyde gave also a crystalline product, which geometric structure was readily determined to be (E)-3-isobutylidene-(Z)-6-benzylidene-PDO [(3E, 6Z)-3] by the comparison of the chemical shifts at δ 7.24 and 5.96 of two exocyclic olefinic protons of the product with those at δ 7.32 and 6.40 of (3Z, 6Z)-3 firstly obtained. These facts and the following results show that the (Z)-geometric arylidene or alkylidene group is stereospecifically formed in the chemical syntheses.[13]



[(3E)-10c] [(3E, 6Z)-3]

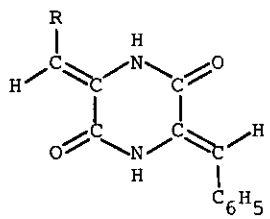
Furthermore, similar work up of 1,4-diacetyl-(E)-3-benzylidene-PDO [(3E)-10c] with isobutyraldehyde gave two geometric isomers as a mixture, one of which could be separated purely by the recrystallization from boiling acetic acid.



[(3E)-10a] [(3Z, 6E)-3] [(3E, 6E)-3]

Surprisingly, from the comparison of the chemical shifts of the olefinic protons, it was found that the desired (E)-3-isobutylidene-(Z)-6-benzylidene- and (E)-3-isobutylidene-(E)-6-benzylidene-PDO [(3Z, 6E)-3 and (3E, 6E)-3] as a mixture, and the latter could be isolated purely. In addition, from the intensity of the olefinic proton signals, the yielding ratio of (3Z, 6E)-3 and (3E, 6E)-3 was determined to be *ca.* 2 : 1.

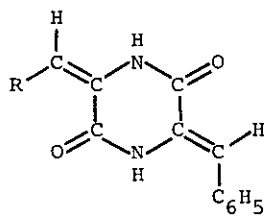
On the other hand, although the compounds 1 and 2 had been prepared previously by the Procedure A or B,[9,37] their configurations have never been confirmed. However, it could be determined to be (Z)-3-(Z)-6-dibenzylidene-PDO and (Z)-3-(p)-anisilidene-(Z)-6-benzylidene-PDO [(3Z, 6Z)-1 and (3Z, 6Z)-2]. It is because that the above two compounds were prepared by the condensation of 1-acetyl-(Z)-3-benzylidene- and (Z)-3-(p)-anisilidene-PDO with benzaldehyde by the Procedure B and the signals of 3- and 6-olefinic protons of 1 and 2 obtained shift at comparatively lower magnetic field (δ 6.82-6.80) than that of 3-olefinic proton (δ 5.96) of (3E, 6Z)-3.



[(3Z, 6Z)-1; R=C_6H_5]

[(3Z, 6Z)-2; R=(p)-CH_3O-C_6H_4]

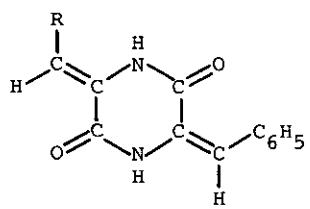
[(3Z, 6Z)-3; R=$CH(CH_3)_2$]



[(3E, 6Z)-1; R=C_6H_5]

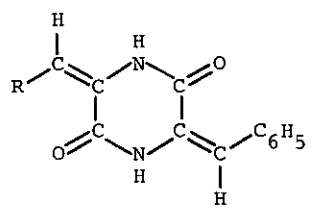
[(3E, 6Z)-2; R=(p)-CH_3O-C_6H_4]

[(3E, 6Z)-3; R=$CH(CH_3)_2$]



[(3Z, 6E)-2; R=(p)-CH_3O-C_6H_4]

[(3Z, 6E)-3; R=$CH(CH_3)_2$]



[(3E, 6E)-3; R=$CH(CH_3)_2$]

Fig. 2

Contrary to expectation, the condensation of the individual (E)-3-benzylidene-PDO [(3E)-7a] and 1,4-diacetyl-(Z)-3-benzylidene-PDO [(3Z)-10a] with benzaldehyde by the Procedure A or B gave the same product as (3Z, 6Z)-1. From the result, it was found that (E)-substituent in the 3-position was readily isomerized under heating. Then, according to the Procedure C, the mild condensation reaction of 1,4-diacetyl-(E)-3-benzylidene [(3E)-10a]- and 1-acetyl-(E)-3-(p)-anisilidene-PDO [(3E)-9b] with benzaldehyde was successful to give the expected (E)-3-(Z)-6-dibenzylidene- and (E)-3-(p)-anisilidene-(Z)-6-benzylidene-PDO [(3E, 6Z)-1 and (3E, 6Z)-2] respectively. On the other hand, the condensation of (3E)-10a with (p)-anisaldehyde gave (Z)-3-(p)-anisilidene-(E)-6-benzylidene-PDO [(3Z, 6E)-2]. The 3- and 6-olefinic protons of (Z)-arylidene group of 1 and 2 obtained above resonate at lower magnetic field ($\Delta\delta$ 0.2 ppm) compared with that of (E)-arylidene and the chemical shifts at δ 6.76-6.72 were very similar to that of (3Z, 6Z)-1 and 2.

The above results suggest that (Z)-substituent is introduced preferentially. Moreover, attempts on the preparation of (3E, 6E)-dibenzylidene-PDO [(3E, 6E)-1] by taking advantage of the steric hindrance of large group, such as benzoyl, e. g., by the reaction of 1,4-dibenzoylglycine anhydride with benzaldehyde by means of the Procedures B and C, failed and gave (3Z, 6Z)-1 alone.

In consequence, the nine geometric isomers of 1, 2, and 3 were first obtained in *ca.* a 60% yield, and the physical constants and the spectral data were listed

Table 5. The yields and melting points of 1, 2, and 3

Compound No.	Procedure	Yield (%)	Mp ^{o}C[a] (decomp.)
(3Z, 6Z)-1	B	73.5	283-284 (295)
(3Z, 6Z)-2	B	60.0	263-265 (270)
(3Z, 6Z)-3	B	58.9	271-271.5
(3E, 6Z)-1	C	69.0	270-272 (281)
(3E, 6Z)-2	C	53.2	252-253 (258)
(3E, 6Z)-3	C	43.8	252-253
(3Z, 6E)-2	C	61.5	253-254 (260)
(3Z, 6E)-3	C	54.6[b]	277-278[c]
(3E, 6E)-3	C	54.6[b]	277-278[c]

a) Colorless or pale yellow crystals from acetic acid. b) Mixture of (3Z, 6E)-3 and (3E, 6E)-3. c) Mixture melting point.

in Tables 5, 6, and 7. Moreover, all the structures of 1, 2, and 3 are illustrated in Fig. 2.

Table 6. The NMR spectral data of 1, 2, and 3

Compound No.	NMR spectrum, δ in DMSO-d_6 or CF_3COOH					
	NH[a]	3-Olefinic (J_{Hz})	6-Olefinic	$(CH_3)_2$	C_6H_4-X $CH(CH_3)_2$	OCH_3
(3Z, 6Z)-1	10.28	6.83(s)	6.82		7.36-7.66(m)[c]	
(3Z, 6Z)-2	10.16	6.80(s)	6.80		6.97-7.63(m)[c]	3.85(s)
(3Z, 6Z)-3	9.76	6.40(d) (10.0)	7.32	1.19(s) 1.25(s)	7.45(s)[d] [2.87(m)]	
(3E, 6Z)-1	10.08 10.85	6.76(s)	6.54		7.18-7.66(m)[c]	
(3E, 6Z)-2	10.02 10.75	6.72(s)	6.52		6.86-7.70(m)[c]	3.81(s)
(3E, 6Z)-3	9.56	5.96(d) (10.0)	7.24	1.12(s) 1.20(s)	7.46(s)[d] [3.76(m)]	
(3Z, 6E)-2	9.97 10.77	6.56(s)	9.74		6.97-7.63(m)[c]	3.85(s)
(3Z, 6E)-3	10.00	6.33(d) (10.0)	6.89	1.16(s) 1.24(s)	7.34(m)[d] [2.83(m)]	
(3E, 6E)-3	10.08	5.87(d) (10.0)	6.83	1.11(s) 1.18(s)	7.42(m)[d] [3.75(m)]	

a) Broad singlet. b) Singlet. c) Measured in DMSO-d_6. d) Measured in CF_3COOH.

Table 7. The IR and UV spectral data of 1, 2, and 3

Compound No.	IR spectrum, cm^{-1} in KBr			UV spectrum, nm in 95% ethanol (log ε)
	NH (w)[a]	NCO (s)[b]	C=C (m)[c]	
(3Z, 6Z)-1	3200	1695	1635	233 (3.95), 338 (4.47)
(3Z, 6Z)-2	3200	1690	1635	236 (3.95), 325 (4.45)
(3Z, 6Z)-3	3182	1690	1640	234 (3.90), 317 (4.33)
(3E, 6Z)-1	3170	1695	1630	232 (3.89), 337 (4.30)
(3E, 6Z)-2	3190	1690	1638	234 (3.93), 352 (4.40)
(3E, 6Z)-3	3175	1682	1638	231 (3.82), 237 (3.80)[d], 317 (4.32)
(3Z, 6E)-2	3170	1682	1625	235 (3.96), 352 (4.47)
(3Z, 6E)-3	3160	1685	1642	232 (3.93), 238 (3.92)[d], 323 (4.30)

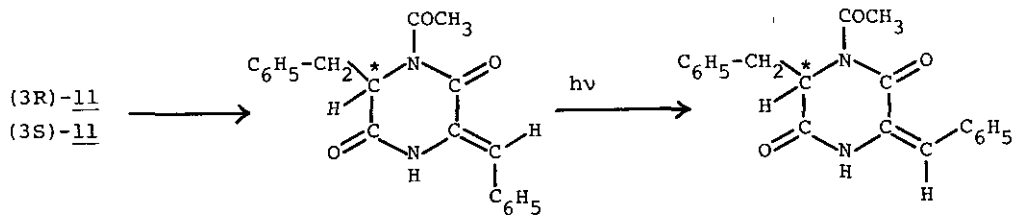
a) Weak. b) Strong. c) Medium. d) Shoulder.

Consequently, it was found that the configurational structure of naturally occurring 1, 2, and 3 could be elucidated unambiguously to be (3Z, 6Z)-geometry, since the melting (decomposition) points, the spectral data of 1, 2, and 3 isolated from the strains were in an excellent agreement with those of (3Z, 6Z)-1, 2, and 3 prepared here, as shown in Tables 5, 6, and 7.

From the above results and facts, concerning the condensation mechanism, it is assumed that (E)-arylidene derivative was formed as an unstable intermediate, followed by the immediate isomerization gave the (Z)-configurational product. These supposition and conclusion will be supported by the facts that the biosynthesis of 3-alkylidene- or 3-arylidene-PDO derivatives, such as mycelianamide,[39] cryptoechinuline A,[40] neoechinuline,[41] by *incorporation of* L-tyrosine *or* L-tryptophan into cyclic dipeptide and subsequent stereoselective dehydrogenation gave predominantly (Z)-isomer.

Furthermore, the similar condensation reaction of various (Z)-3-, (E)-3-alkylidene-PDO, 1-acetyl-, 1,4-diacetyl-(Z)-3- and (E)-3-alkylidene-PDO with an appropriate aldehyde gave a number of unsymmetric unsaturated PDO derivatives in good yields.

On the other hand, treatment of the individual (3R)-11 and (3S)-11 with benzaldehyde in DMF in the presence of t-BuOK/t-BuOH at 0 °C for 12 hours gave the desired 4-acetyl-(R)-3- and (S)-3-benzyl-(Z)-6-benzylidene-PDO [(3R, 6Z)-12 and (3S, 6Z)-12] in good yields respectively. Subsequently, the photoisomerization of the (Z)-isomers thus obtained into the corresponding (E)-isomers was performed, according to the method of Blake and Sammes.[34] Irradiation of the (3R, 6Z)-12 and (3S, 6Z)-12 in ethanol with 500 W high pressure mercury lamp under nitrogen gas at room temperature for 5 hours gave the corresponding (E)-



[(3R, 6Z)-12]
[(3S, 6Z)-12]

[(3R, 6E)-12]
[(3S, 6E)-12]

isomers [(3R, 6E)- and (3S, 6E)-12] in *ca.* 50% yield respectively.

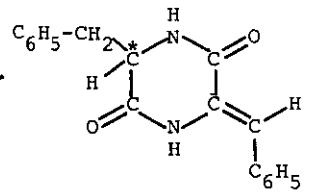
Table 8. The yields and the specific rotations of 12

Compound No.	Yield (%)	Appearance[a)]	$[\alpha]_D^{25}$ in EtOH
(3R, 6Z)-12	92	syrup	-483° (*c* 1.2)
(3R, 6E)-12	50	syrup	259° (*c* 1.2)
(3S, 6Z)-12	89	syrup	558° (*c* 1.2)
(3S, 6E)-12	51	syrup	-239° (*c* 1.2)

a) Colorless.

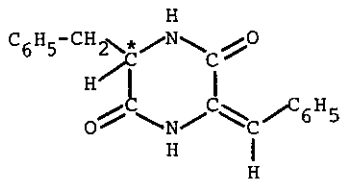
Furthermore, the deacetylation of (3R, 6Z)- and (3S, 6Z)-12 with hydrazine hydrate in DMF under cooling for 1 hour gave the expected (R)-3- and (S)-3-benzyl-(Z)-6-benzylidene-PDO [(3R, 6Z)-4 and (3S, 6Z)-4] in good yields respectively. Similar treatment of (E)-isomers of 12 was worked up to give the corresponding deacetylated products [(3R, 6E)-4 and (3S, 6E)-4 in *ca.* 90% yields respectively.

(3R, 6Z)-12
(3S, 6Z)-12 ⟶



[(3R, 6Z)-4]
[(3S, 6Z)-4]

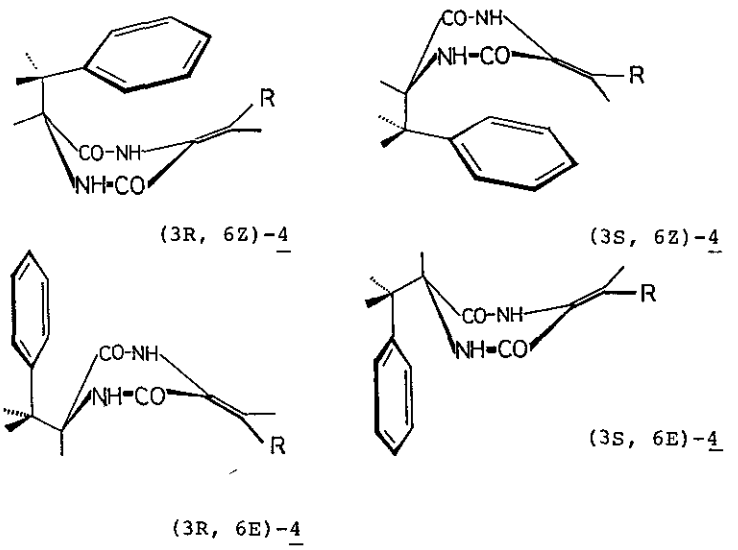
(3R, 6E)-12
(3S, 6E)-12 ⟶



[(3R, 6E)-4]
[(3S, 6E)-4]

From the above results, very interesting phenomena were observed in the specific rotation and the CD spectrum of 4 and 12. Surprisingly, the sign of

the optical rotation and the Cotton effect were reversed not only between each pair of (R)- and (S)-isomers, but also that of (Z)- and (E)-isomers, as is summarized in Tables 8 and 10. These facts may imply the transformation of the conformation,[41,42] for example, flagpole half boat and bowsprit half boat, depending on the (Z)- or (E)-configuration at C-6, as is illustrated in Fig. 3. Further work including the proof of the above deduction is now in progress.



(3R, 6Z)-4 (3S, 6Z)-4

(3S, 6E)-4

(3R, 6E)-4

Fig. 3

Table 9. The yields and melting points of 4

Compound No.	Yield (%)	Mp °C
(3R, 6Z)-4	78	273.5-274.5
(3R, 6E)-4	75	260.0-261.5
(3S, 6Z)-4	73	271.5-272.5
(3S, 6E)-4	76	259.0-260.5

Table 10. The spectral data of 4

Compound No.	NMR spectrum, δ in CF_3COOH NH (s)	-CH=(s)	3-proton	$[\alpha]_D^{25}$ [a]	UV, nm (log ε) in 95% EtOH
(3R, 6Z)-4	8.96 8.48	6.97	4.80m	-550°	295 (4.10)
(3R, 6E)-4	9.68 8.30	6.54	4.80m	360°	302 (4.06)
(3S, 6Z)-4	9.00 8.44	6.97	4.82m	440°	294 (4.02)
(3S, 6E)-4	9.60	6.54	4.80m	-320°	305 (4.08)

a) Recorded in ethanol (c 1.2).

In conclusion, from the comparison of the physical properties of naturally occurring 3-benzyl-6-benzylidene-PDO (mp 288.5-290.0 °C, $[\alpha]_D$ -520°, IR: 3189, 1661, and 1626 cm^{-1}, UV: 296 nm (log ε=4.10) with those of four isomers of 4 indicated that (3R, 6Z)-4 (IR: 3190, 1680, 1665, and 1627 cm^{-1}) was identical with the natural product.

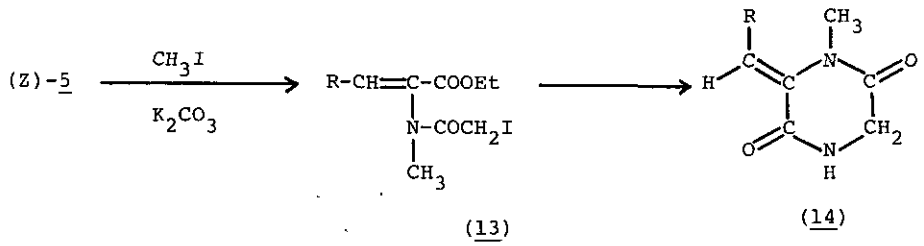
6. Methylation of PDO with Methyl Iodide

Recently, many bioactive oligopeptides N-blocked partially with methyl group have been discovered. Furthermore, antibioactive N-methylated dehydropeptides, such as tentoxin,[43] were isoalted, a few of which were synthesized.[44,45,46] The useful N-methylation of α-amino acid and its residue in peptide has been almost established by the several methods,[47-50] but that of α-dehydroamino acid (DHA) and dehydropeptide (DHP) has not yet been investigated thoroughly. Because of more lability of DHA and DHP and of restriction, e. g., hydrolysis and geometric isomerization,[51] under currently employed procedures, it is necessary to establish the optimal conditions for the N-methylation of the various DHA and DHP.

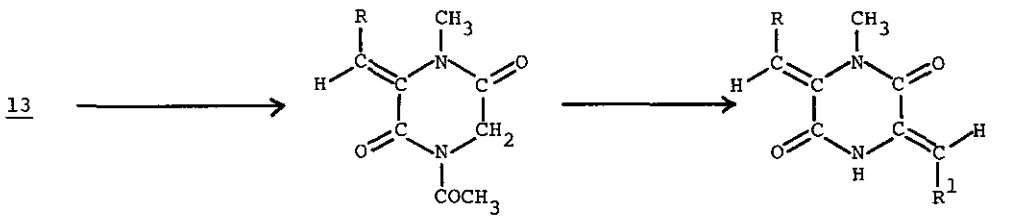
Various routes for the selective methylation at 1-, 4-, and 1,4-positions , of 3- and 3,6-dialkylidene-PDO by the reaction with methyl iodide followed by the cyclization of DHA with methylamine were reported.[18] Moreover, 1-, 4-, and 1,4-dimethylated albonoursin and its two analogs were also synthesized.

The reaction of haloacetyl-DHA (5) with 5 moles of methyl iodide and K_2CO_3 in DMF at room temperature for 48 hours was achieved to give the desired

N-methylated DHA esters (13) in *ca.* a 84% yield. In this case, it was found that the halogen exchange between chlorine and iodine occurred to give the corresponding (Z)-2-(N-methyl)iodoacetyl derivative (13). Subsequently, according to the usual method,[52] the compound 13 obtained was cyclized with gaseous ammonia in methanol to give colorless crystals, identified as 4-methyl-(Z)-3-alkylidene-PDO (14) in a 71% yield.

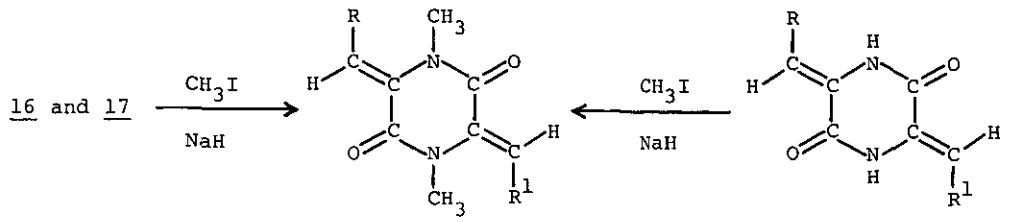


Furthermore, for the condensation of 14 with aldehyde, the acetylation of 14 with acetic anhydride was carried out under reflux for 2 hours to give 1-acetyl-4-methyl-(Z)-3-alkylidene-PDO (15) in an almost quantitative yield. The condensation of 15 with 5 moles of an appropriate aldehyde in t-butanol in the presence of 0.5 M potassium t-butoxide gave 4-methyl-(Z)-3-(Z)-6-dialkylidene-PDO (16 and 17) as colorless crystals in *ca.* a 52% yield. Further methylation of the individual 16 and 17 with methyl iodide and sodium hydride was worked up similarly to give the corresponding 1,4-dimethyl derivative (18), by the usual way.[53]



On the other hand, albonoursin [(3Z, 6Z)-3] and the other naturally occurring analogs [(3Z, 6Z)-1 and (3Z, 6Z)-2] synthesized were subjected to the direct methylation. *The individual 1, 2, and 3 reacted readily with methyl iodide in* the presence of sodium hydride at room temperature for 1 hour to give the expected

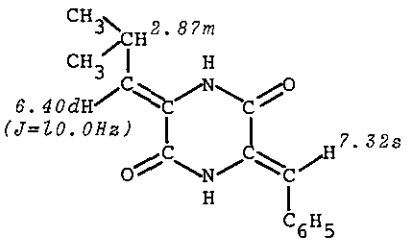
18 in *ca.* a 73% yield, which was in complete agreement with the product obtained from 16 and 17 and methyl iodide.



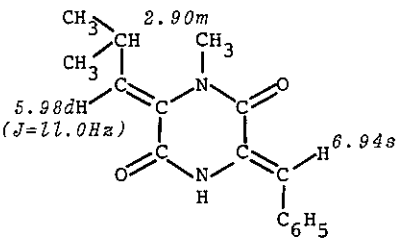
(18) (1, 2, and 3)

In consequence, the structurally interesting 1-, 4-, and 1,4-dimethyl derivatives of albonoursin and its two analogs could be prepared here.

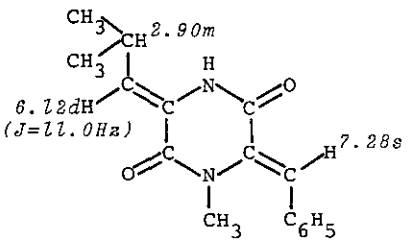
The yields, melting points, and spectral data of 16, 17, and 18 are listed in Table 11. Especially, in order to compare two olefinic and methine protons between albonoursin and the other N-methylated analogs, the structure of four compounds is illustrated in Fig 4.



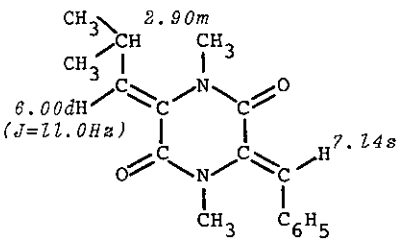
Albonoursin



(16)



(17)



(18)

Fig. 4

Table 11. The yields, melting points, and NMR spectral data of 16-17

Compound No.	R	R^1	Yield (%) A[a]	Yield (%) B[b]	Mp °C[e]	NMR, δ in $CDCl_3$ $N-CH_3$	NMR, δ in $CDCl_3$ NH
16	i-C_3H_7	C_6H_5	51		142-143	3.38	8.30
17	C_6H_5	i-C_3H_7	52		165-166	2.99	9.70
	i-C_3H_7	C_6H_5	62[c] 74[d]	72	125-126	2.90 3.37	——
18	C_6H_5	C_6H_5		77	138-139	3.00	——
	(p)-$CH_3O-C_6H_4$	C_6H_5		70	140-142	3.00 3.05	——

a) Yield from 16 and 17. b) Yield from 1, 2, and 3. c) Yield from 16.
d) Yield from 17. e) Colorless needles.

7. Conclusion

It is believed firmly that the synthesis and the reaction of exocyclic unsaturated and optically active PDO will contribute to the development of the chemistry of recent interesting dehydropeptides, which are very important for the investigation on the correlation between the structure and bioactivity. In particular, the criterion obtained from the NMR spectral data accumulated in our recent works may be applicable to the configurational determination of the other unsaturated cyclic peptide and dehydrooligopeptide.

Moreover, it is also very attractive to the asymmetric hydrogenation and asymmetric addition of unsaturated and optically active PDO,[52] containing dehydrooligopeptides. Therefore, 3- and 3,6-disubstituted unsaturated PDO must be utilized as the important substrate for the asymmetric reactions and the synthesis of naturally occurring products, derived from α-amino acid and α-dehydroamino acid.

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