Configuration of echinulin. II. Optical rotatory dispersion of echinulin, hydroechinulin, and the stereoisomeric 3-methyl-6(indolyl-3-methyl)-piperazine-2,5-diones

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Configuration of echinulin. II.[1] Optical rotatory dispersion of echinulin, hydroechinulin, and the stereoisomeric 3-methyl-6(indolyl-3-methyl)-piperazine-2,5-diones[2,3]

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The optical rotatory dispersion spectra of echinulin and hydroechinulin have been compared with the spectra of the 4 stereoisomers of *cyclo*-alanyltryptophan. The results indicate appreciable differences in the spectra of the *cis*- and *trans*-piperazinediones and that echinulin has the *cyclo*-L-alanyl-L-tryptophan configuration.

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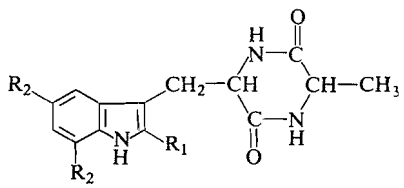
Echinulin (**1***a*) is a white crystalline compound which was first isolated from a culture of *Aspergillus echinulatus* (Delacr) Thom & Church (1). The structure (**1***a*) was established by a series of degradations and partial synthesis (2) and is supported by nuclear magnetic resonance (n.m.r.) (3) and biochemical (4) studies.

Acid hydrolysis (5) of hexahydroechinulin (hydroechinulin) (**1***b*) gave L-alanine and the amino acid hexahydroechinin (hydroechinin) (**2***b*). Echinulin (**1***a*) may be regarded then as a piperazinedione formed by the condensation of L-alanine and the amino acid echinin (2′-(1,1-dimethylprop-2-enyl)-5′,7′-di-(3-methylbut-2-enyl)-tryptophan) (**2***a*) of unknown configuration.



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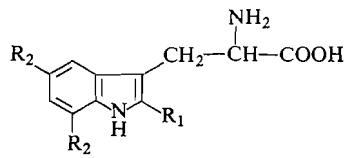
(*a*) $R_1 = -C(CH_3)_2-CH=CH_2$;
$R_2 = -CH_2-CH=C(CH_3)_2$

It has been found (6) that the absolute configuration of amino acids is indicated by their optical rotatory dispersion (o.r.d.) between 200 and 225 mμ. The configuration of echinin (**2***a*) could presumably be determined in this way also.



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(*b*) $R_1 = -C(CH_3)_2-CH_2CH_3$;
$R_2 = -CH_2-CH_2-CH(CH_3)_2$

However, echinin (**2***a*) could not be obtained (5) by acid hydrolysis of echinulin (**1***a*) although hydroechinulin (**1***b*) gave hydroechinin (**2***b*) under the same conditions. The acid hydrolysis of hydroechinulin was previously used in this laboratory in connection with biosynthetic studies (7) but did not give a pure product. In view of this, and the observation (8) that the configuration of a piperazinedione can be determined by o.r.d., it was decided to compare the o.r.d. spectra of echinulin and the model *cyclo*-alanyltryptophans (**1**, $R_1 = R_2 = H$).

The piperazinediones were prepared by the cyclization (9) of alanyltryptophan or tryptophylalanine as shown in Fig. 1. In practice it was found more convenient to use the latter compound, as the synthesis of benzyloxycarbonyltryptophan presented less difficulty than the synthesis of tryptophan benzyl ester. Our investigations showed that the intermediate protected dipeptides obtained by the carbodiimide method (10) were identical in m.p. and specific rotation with the corresponding compounds prepared by the azide method (11). The various intermediates were obtained in good yield but since the carbodiimide method requires fewer steps than the azide method, the preferred synthesis of *cyclo*-alanyltryptophan is that shown in Fig. 1, route A.

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[1]For Part I, see ref. 14.

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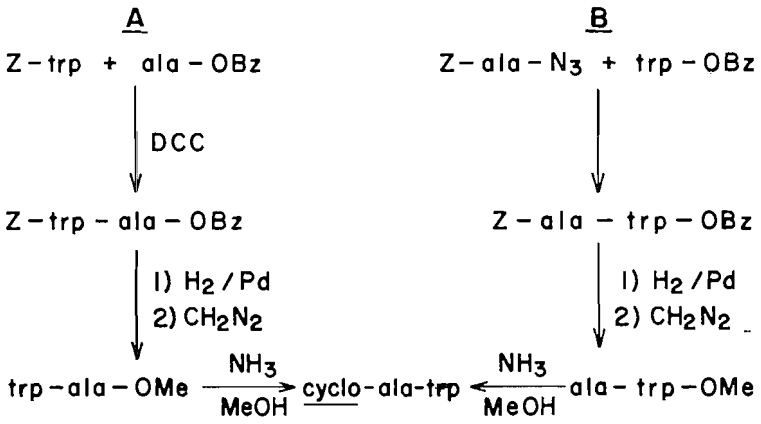

FIG. 1. Synthesis of *cyclo*-alanyltryptophan. Z = benzyloxycarbonyl, DCC = *N,N'*-dicyclohexylcarbodiimide.

Since this work was completed, two methods of preparing sterically pure piperazinediones have been reported (12, 13). A number of piperazinediones synthesized by Fischer's method (9) were found (13) to contain varying amounts of racemic material. However, thin-layer chromatography (t.l.c.) (13) of the piperazinediones used in the present work did not indicate racemization. The racemization may have been avoided by using shorter reaction times (5 h) than those (1–5 days) used by Nitecki *et al.* (13).

In a preliminary communication (14) the o.r.d. spectra of echinulin in chloroform and hydroechinulin in ethanol were compared with the o.r.d. spectra of *cyclo*-L-alanyl-L-tryptophan and *cyclo*-L-alanyl-D-tryptophan in diglyme–water (4:1) from 700–300 mμ. The similarity of the curves for *cyclo*-L-alanyl-D-tryptophan, echinulin, and hydroechinulin prompted the suggestion that the tryptophan moiety in echinulin has the D-configuration. The spectra of these compounds and the other stereoisomeric piperazinediones, *cyclo*-D-alanyl-D-tryptophan, and *cyclo*-D-alanyl-L-tryptophan, in ethanol are presented in Figs. 2–5. In the 650–350 mμ region these compounds possess plain dispersion curves as previously described (14). Below 350 mμ the spectra are more complex and more valuable in determining configuration, and only spectra covering this region have been reproduced. The spectra of the piperazinediones (Figs. 2–5) are divided into two parts (200–250 mμ and 230–320 mμ) with the ordinates in the ratio of 10:1. This method of presentation has been used because of the large differences in the molecular rotations and

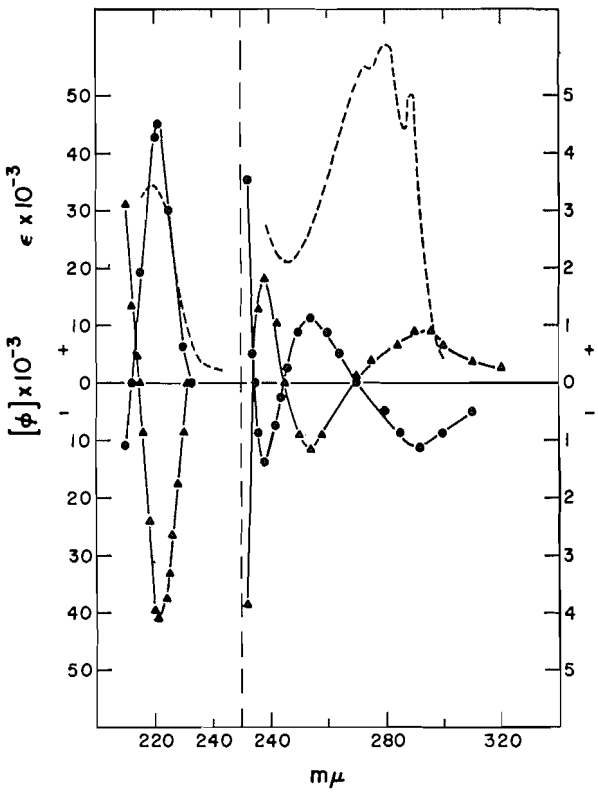

FIG. 2. Optical rotatory dispersion spectra of *cyclo*-L-alanyl-L-tryptophan (▲) and *cyclo*-D-alanyl-D-tryptophan (●) in ethanol. Ultraviolet spectrum in ethanol (– – –).

ultraviolet (u.v.) absorptions in these two regions. The spectra of echinulin (Fig. 4) and hydroechinulin (Fig. 5) have likewise been presented in two parts (200–280 mμ and 230–320 mμ)

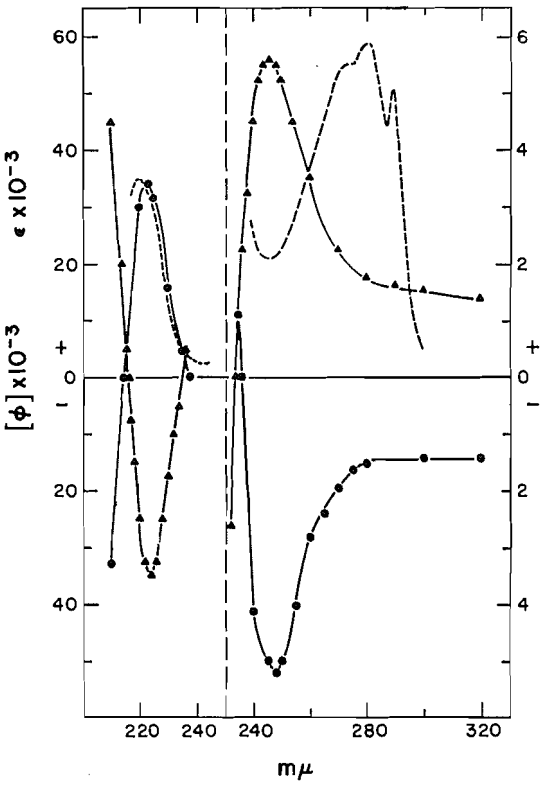

FIG. 3. Optical rotatory dispersion spectra of *cyclo*-D-alanyl-L-tryptophan (▲) and *cyclo*-L-alanyl-D-tryptophan (●) in ethanol. Ultraviolet spectrum in ethanol (– – –).

which allow for comparison with the spectra of the piperazinediones.

The o.r.d. spectra of the piperazinediones, echinulin, and hydroechinulin, show a number of differences in the 350–230 mμ region (cf. Figs. 2–5). The absence of a complex Cotton effect at 230–320 mμ in the spectra of the *trans*-piperazinediones may be due to the extrema near 246 mμ (Fig. 3) which mask the weak Cotton effects shown by the *cis*-piperazinediones (Fig. 2) in this region (the rotations of the *trans* compounds are almost three times the rotations of the *cis* compounds at these wavelengths). Echinulin (Fig. 4) and hydroechinulin (Fig. 5) also do not possess complex Cotton effects at 300–230 mμ but have instead a rather broad Cotton effect. The rotations of echinulin, hydroechinulin, and the *cis*-alanyltryptophans are of similar magnitude and the difference in the complexity of the spectra may be related to the steric effects of the alkyl substituents of echinulin and hydroechinulin.

Proton magnetic resonance (p.m.r.) studies (15) indicate that the preferred conformation of *cyclo*-alanylphenylalanine is such that the benzene and piperazinedione rings face each other. A similar folding would be expected of the *cyclo*-alanyltryptophans. Construction of models (Corey–Pauling–Koltun) (16) indicates that in the *cis* compounds, the indole nucleus can lie over the piperazinedione ring so that a hydrogen atom of the alanine methyl group is close to the mid-point of the bond common to the five- and six-membered rings (a model of *cis*-alanylphenylalanine shows that the corresponding hydrogen atom is near the center of the benzene ring). A model of echinulin in the folded form is very difficult to construct because of interference from the alkyl substituent in the α-position of the indole nucleus. The strain is not affected by placing the alanine methyl group in the *trans* position but is relieved by swinging the indole nucleus away from the

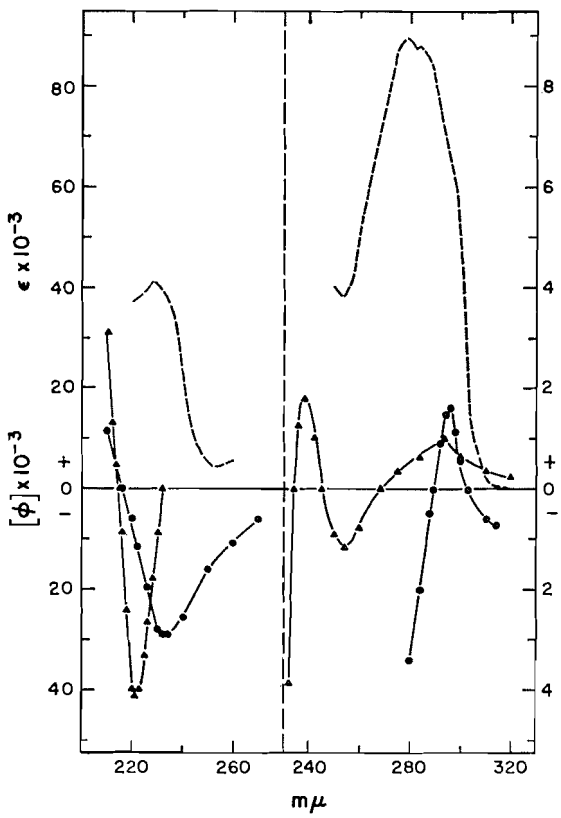

FIG. 4. Optical rotatory dispersion spectra of echinulin (●) and *cyclo*-L-alanyl-L-tryptophan (▲) in ethanol. Ultraviolet spectrum of echinulin in ethanol (– – –).

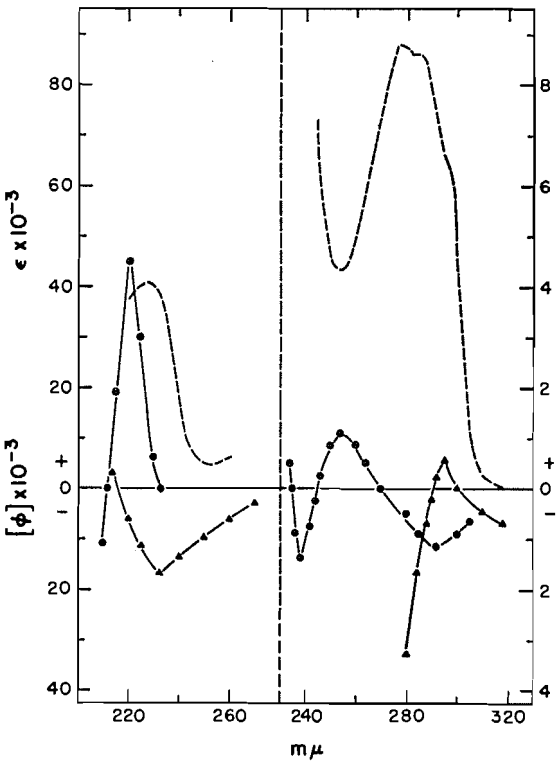

FIG. 5. Optical rotatory dispersion spectra of hydroechinulin (▲) and *cyclo*-D-alanyl-D-tryptophan (●) in ethanol. Ultraviolet spectrum of hydroechinulin in ethanol (– – –).

piperazinedione ring. Thus the molecular models indicate that interaction between the π-electrons of the indole and carbonyl groups would be expected to decrease in the order *trans-cyclo*-alanyltryptophan > *cis-cyclo*-alanyltryptophan > echinulin. Klyne *et al.* (17) have shown that the Cotton effects at 250–230 mμ of the heteroyohimbine alkaloids are influenced by the relative positions of the indole and carbomethoxy groups. Other examples of Cotton effects being related to the interaction of two π-electron systems are also known (18), and the results of the present study may be another instance of this type of relationship.

The o.r.d. spectra of echinulin (Fig. 4) and hydroechinulin (Fig. 5) possess the same broad positive Cotton effect in the 300–220 mμ region. These spectra are compared with the spectra of *cyclo*-L-alanyl-L-tryptophan (Fig. 4) and *cyclo*-D-alanyl-D-tryptophan (Fig. 5), respectively. The trough of the Cotton effects of echinulin and hydroechinulin are at longer wavelength (232 mμ) than the trough of the Cotton effect of *cyclo*-L-alanyl-L-tryptophan (221 mμ) (Fig. 4), or the corresponding trough of *cyclo*-D-alanyl-L-tryptophan (224 mμ) (Fig. 3). However, the troughs of these Cotton effects coincide with the relevant u.v. absorption maxima (cf. Figs. 2–5) and would thus appear to be related to the same chromophores. Comparison of the o.r.d. spectra of hydroechinulin and *cyclo*-D-alanyl-D-tryptophan (Fig. 5) shows that the latter has Cotton effects of opposite sign to the former. The o.r.d. spectra below 300 mμ therefore indicate that echinulin has the *cyclo*-L-alanyl-L-tryptophan configuration, and not the *cyclo*-L-alanyl-D-tryptophan configuration as previously suggested (14). This conclusion is supported by a recent o.r.d. study (19) of two *cyclo*-alanyltryptophans carrying a 1,1-dimethylprop-2-enyl substituent in the α-position of the indole nucleus (**1**, R_1 = —C$(CH_3)_2CH{=}CH_2$, R_2 = H).

The configuration of echinulin has also been investigated (20) by the t.l.c. method used to determine the steric purity of piperazinediones (13). Nitecki *et al.* (13) found that *cis*-piperazinediones have a lower R_f value than the corresponding *trans* compounds. Echinulin and *cyclo*-L-alanyl-L-tryptophan were racemized separately in boiling ethanol–triethylamine and the products, starting materials, and *cyclo*-L-alanyl-D-tryptophan were examined by t.l.c. The results (20) indicated that echinulin behaves like a *cis*-piperazinedione, and since the alanine portion has the L-configuration it follows that the tryptophan moiety must also have the L-configuration. Racemization experiments using the above-mentioned compounds and hydroechinulin gave results identical to those of Westley *et al.* (20).

Experimental

The optical rotatory dispersion (o.r.d.) measurements were made on a Jasco ORD/UV-5 spectropolarimeter. Optical rotations at the sodium-D line were measured at 25° on a Perkin–Elmer141 polarimeter. Ultraviolet absorption spectra were obtained with a Beckman DK-2 spectrometer. Melting points were determined on a Leitz hotstage and are uncorrected. Echinulin and hydroechinulin were obtained as previously described (7). The amino acids were obtained from the Sigma Chemical Company and had specific rotations in agreement with literature values and were used as supplied. The protected amino acids were synthesized by known methods and those previously described had melting points and specific rotations corresponding to the published values. The following derivatives do not appear to have been described previously: D-alanine benzyl ester benzene sulfonate (21), m.p.

120–121° (EtOH–Et_2O), $[\alpha]_D$ −8.1° (*c*, 3.1 pyridine); benzyloxycarbonyl-L-tryptophan hydrazide (24), m.p. 210–212° (EtOH–EtOAc), $[\alpha]_D$ −9.7° (*c*, 1.27 HOAc); D-isomer, m.p. 209–211°, $[\alpha]_D$ +9.2° (*c*, 1.31 HOAc); D-alanine ethyl ester hydrochloride (25*b*), m.p. 75–76° (EtOH–Et_2O), $[\alpha]_D$ +11.1° (*c*, 2.1 in 5 *N* HCl); D-tryptophan benzyl ester hydrochloride (23), m.p. 213–215° (EtOH–Et_2O), $[\alpha]_D$ −4.1° (*c*, 2.1 MeOH).

Benzyloxycarbonyltryptophylalanine Benzyl Ester

N,N'-dicyclohexylcarbodiimide (1.36 g, 0.006 mole) (23) was added to a mixture of benzyloxycarbonyltryptophan (22) (2.0 g, 0.0059 mole), alanine benzyl ester benzenesulfonate (21) (2.0 g, 0.0059 mole), and triethylamine (0.85 ml, 0.006 mole) in dichloromethane (25 ml). After stirring at room temperature for 4 h, acetic acid (0.2 ml) was added and the mixture filtered to remove dicyclohexylurea. The filtrate was washed with 0.5 *N* HCl, 0.5% $NaHCO_3$, and water. Evaporation of the dried ($MgSO_4$) solution left a mixture of dicyclohexylurea and an oil which was taken up in acetone and filtered. Addition of petroleum ether to the filtrate, and cooling, gave the product in 70–75% yield.

Benzyloxycarbonyl-L-tryptophyl-L-alanine benzyl ester, m.p. 150–152°, $[\alpha]_D$ −28.4° (*c*, 0.54 MeOH) (lit. (23) m.p. 153°, $[\alpha]_D^{25}$ −27° (*c*, 1 MeOH)).

Benzyloxycarbonyl-D-tryptophyl-D-alanine benzyl ester, m.p. 146–149°, $[\alpha]_D$ +27.4° (*c*, 0.58 MeOH).

Benzyloxycarbonyl-L-tryptophyl-D-alanine benzyl ester, m.p. 163–164°, $[\alpha]_D$ +9.8° (*c*, 0.59 MeOH).

Benzyloxycarbonyl-D-tryptophyl-L-alanine benzyl ester, m.p. 161–163°, $[\alpha]_D$ −9.2° (*c*, 0.72 MeOH).

Anal. Calcd. for $C_{29}H_{29}N_3O_5$: C, 69.7; H, 5.9; N, 8.4. Found: C, 69.5; H, 5.9; N, 8.7.

Coupling of benzyloxycarbonyltryptophan hydrazide (24) and alanylbenzyl ester (21) by the azide method (24) gave products identical to those from the carbodiimide method, although in lower yield (50–55%).

Cyclo-alanyltryptophan

A stream of hydrogen was passed through a stirred solution of benzyloxycarbonyltryptophylalanine benzyl ester (0.56 g, 0.0011 mole) in methanol (50 ml) containing palladium black (approximately 0.1 g) until carbon dioxide was no longer evolved (25*a*). If free peptide precipitated during hydrogenolysis it was dissolved by addition of water. The reaction mixture was filtered and the filtrate evaporated to dryness under reduced pressure at 50° to give tryptophylalanine as a white solid (90–95%) which was chromatographically pure (23). The tryptophylalanine tended to decompose (judged by the development of a yellow-brown color) when subjected to recrystallization procedures and the product obtained from hydrogenolysis was used directly in the following step.

The alanyltryptophan in methanol (50 ml) at room temperature was treated with an ether solution of diazomethane over 1 h. After stirring for a further 1 h the solvent was removed under reduced pressure and the residue dissolved in methanol (100 ml). The solution was cooled to 0° and saturated with dry ammonia gas (9). After stirring for 5 h the solvent was removed under reduced pressure at 40° and the product crystallized from ethanol – ethyl acetate in yields of 60–65%.

Cyclo-L-alanyl-L-tryptophan, m.p. 282–284° (decomp.) $[\alpha]_D$ +10.4°, (*c*, 0.48 EtOH).

Cyclo-D-alanyl-D-tryptophan, m.p. 281–283° (decomp.) $[\alpha]_D$ −10.2° (*c*, 0.048 EtOH).

Cyclo-D-alanyl-L-tryptophan, m.p. 265–267° (decomp.) $[\alpha]_D$ +75.6° (*c*, 0.049 EtOH).

Cyclo-L-alanyl-D-tryptophan, m.p. 264–267° (decomp.) $[\alpha]_D$ −74.2° (*c*, 0.051 EtOH).

λ_{max} (EtOH) 220 (35 700), 274 (5 790), 280 (5 870), 290 (5 080), mμ.

Anal. Calcd. for $C_{14}H_{14}N_3O_2$: C, 65.4; H, 5.9; N, 16.3. Found: C, 65.2; H, 5.8; N, 16.1.

Benzyloxycarbonylalanyltryptophan Benzyl Ester

Benzyloxycarbonylalanine hydrazide (24) (2.43 g, 0.0103 mole) in a mixture of acetic acid (12 ml), water (50 ml) and 5 *N* hydrochloric acid (5 ml) was treated with a solution of sodium nitrite (0.8 g, 0.0116 mole) in water (2.5 ml) as previously described (24). The dry ether solution of the azide was added to a solution of tryptophan benzyl ester (prepared by treating the hydrochloride (3.4 g, 0.0103 mole) in chloroform (20 ml) with triethylamine (5 ml, 0.0358 mole) at room temperature for 20 min, filtering, and evaporating to dryness). After standing at room temperature overnight, the reaction mixture was worked up in the usual way (24) and the solid product recrystallized from acetone – petroleum ether in yields of 75–80%.

Benzyloxycarbonyl-L-alanyl-L-tryptophan benzyl ester, m.p. 105–106°, $[\alpha]_D$ −23.2° (*c*, 1.1 MeOH) (lit. (23) m.p. 105°, $[\alpha]_D^{25}$ −22° (*c*, 1 MeOH)).

Benzyloxycarbonyl-D-alanyl-D-tryptophan benzyl ester, m.p. 104–105°, $[\alpha]_D$ +20.2° (*c*, 1.2 MeOH).

Benzyloxycarbonyl-D-alanyl-L-tryptophan benzyl ester, m.p. 158–160°, $[\alpha]_D$ +12.8° (*c*, 0.57 MeOH).

Benzyloxycarbonyl-L-alanyl-D-tryptophan benzyl ester, m.p. 158–160°, $[\alpha]_D$ −12.4° (*c*, 0.46 MeOH).

Anal. Calcd. for $C_{29}H_{29}N_3O_5$: C, 69.7; H, 5.9; N, 8.4. Found: C, 69.5; H, 5.8; N, 8.5.

Products identical to the above were obtained in 75–80% yield by treating benzyloxycarbonylalanine (26) and tryptophan benzyl ester with dicyclohexylcarbodiimide (23).

Cyclo-alanyltryptophan

Benzyloxycarbonylalanyltryptophan benzyl ester (2 g, 0.004 mole) in methanol (50 ml) containing palladium black (0.5 g) was hydrogenolyzed (25*a*) as for the benzyloxycarbonyltryptophylalanine benzyl ester to give alanyltryptophan in 90% yield. The peptide was methylated as before and the oily product treated with dry ammonia gas (9) as for the tryptophylalanine, to give *cyclo*-alanyltryptophan in yields of 65–70%. The piperazinediones prepared by this method were identical to the corresponding compounds synthesized from tryptophylalanine.

Racemization and Thin-Layer Chromatography

Samples (25 mg) of echinulin, hydroechinulin, and *cyclo*-L-alanyl-L-tryptophan in ethanol–triethylamine (1:1, 10 ml) were boiled under reflux for 3 days (20). The solvent was removed under reduced pressure and the residues, starting materials, and *cyclo*-L-alanyl-D-tryptophan examined by t.l.c. on silica gel G using diisopropyl ether – chloroform – acetic acid (6:3:2) (Nitecki *et al.* (13) used the ratio 6:3:1 but this was not sufficiently polar for our purpose). The spots were detected by exposing the air-dried plates to iodine vapor, which was

superior to the method of Nitecki *et al.* (13) for the compounds in this study.

Acknowledgments

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