[CONTRIBUTION FROM THE DEPARTMENT OF MICROBIOLOGY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

Synthesis of Peptides Related to Gramicidin S. IV.[1] Two Polypeptide Intermediates Containing L-Phenylalanine in Place of D-Phenylalanine[2]

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A hexapeptide and a tetrapeptide derivative have been synthesized using methods which are designed to avoid diastereoisomer formation. These compounds are intermediates for the synthesis of the acyclic analog of gramicidin S containing L-phenylalanine in place of D-phenylalanine.

As part of the program to synthesize analogs of the polypeptide antibiotic, gramicidin S, the synthesis of the acyclic peptide H·Val-Orn-Leu-Phe-Pro-Val-Orn-Leu-Phe-Pro·OH(10L)[3] is planned. This compound differs from gramicidin S in two ways: (a) it is acyclic and (b) its phenylalanine residues are of the L-configuration rather than the "unnatural" D-configuration. Antibacterial assay of this decapeptide may shed light on the part played by the D-phenylalanine residues in the antibacterial activity of gramicidin S.

This paper reports the synthesis of two intermediates necessary for the preparation of the decapeptide. One is a tetrapeptide derivative: Z. Val-*p*-Tos·Orn-Leu-Phe·NH·NH_2(4 L) I; the other is a hexapeptide derivative: H·Pro-Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe·HCl(6 L) (II). As in the synthesis of the earlier polypeptides,[1] the azide method was utilized whenever diastereoisomer formation was to be avoided. Figure 1 shows the synthetic scheme.

δ-*p*-Toluenesulfonyl L-ornithine benzyl ester was isolated as both the phosphate and the hydrochloride salts. The former compound is, as far as we know, the only amino acid ester phosphate ever described and appeared quite by accident after esterification, using polyphosphoric acid as the condensing agent.[4] Addition of three volumes of ether to the reaction mixture prior to an anticipated treatment with HCl gas resulted in the precipitation of the phosphate salt. In order to prepare the hydrochloride, only one volume of ether could be added before HCl treatment. Subsequently, an additional five volumes of ether were added to precipitate the hydrochloride. Either salt could be used in further synthetic steps.

The tripeptide intermediate, Z·Leu-Phe-Pro·OMe(3 L) (compound 10), was obtained as two fractions: a crystalline product and an oil. Both fractions could be hydrogenated to H·Leu-Phe-Pro·OMe·HCl(3 L) (compound 11), but the lower yield from the non-crystalline product indicated the presence of an impurity. We believe this impurity to be Z·Leu-Phe·NH_2(2 L), on the basis of experiments carried out on some fractions isolated after the hydrogenation of Z·Leu-Phe-Pro·OMe-(L-D-L), a non-crystalline intermediate used in the preparation of the decapeptides in papers I and III of this series. In these earlier experiments, Z·Leu-Phe·NH·NH_2(L-D) was converted, *via* the azide, to Z·Leu-Phe-Pro·OMe(L-D-L). The crude tripeptide derivative could not be crystallized and was hydrogenated as such. Fractional recrystallization of the hydrogenated product yielded two compounds: H·Leu-Phe-Pro·OMe·HCl(L-D-L) and a dipeptide devoid of proline, subsequently identified as H·Leu-Phe·NH_2·HCl(L-D). The latter must have arisen by hydrogenation of Z·Leu-Phe·NH_2(L-D), which was present as an impurity in the non-crystalline product of the azide reaction. That this amide could have been the result of a rearrangement of Z·Leu-Phe·N_3(L-D) is suggested by the finding of Prelog and Wieland[5] that dicarbobenzyloxy L-lysine azide, when used to prepare lysine-containing peptides, yields some dicarbobenzyloxy L-lysine amide. Our conclusion that the oily fraction of compound 10 contains Z·Leu-Phe·NH_2(2 L) as an impurity, follows from analogous reasoning.

The tetrapeptide and hexapeptide derivatives, whose synthesis is described in this paper, will be used to prepare the "10 L" decapeptide analog of gramicidin S.

Experimental[6]

1. δ-*p*-Toluenesulfonyl-L-ornithine Benzyl Ester Phosphate.—27.6 g. (0.0965 mole) of δ-*p*-toluenesulfonyl-L-ornithine (ref. 1, paper I) was dissolved in 150 ml. of benzyl alcohol containing 45 g. of polyphosphoric acid. The solution was stirred for 4 hr. in an oil-bath at 94–96°. At the end of this time it was cooled to room temperature and 300 ml. of anhydrous ether added. After standing several minutes a heavy, white, crystalline precipitate appeared. After refrigeration, the product was filtered off and washed with dry ether; yield 45.7 g., m.p. 185–189°. Recrystallization from 750 ml. of 80% methanol gave 29.5 g. (65%), m.p. 196–197°, $[\alpha]^{25}D$ +5.4 (0.5%) in 0.1 *N* hydrochloric acid (calcd. as free ester). A second recovery of 3.9 g. (74% total yield), m.p. 191–193°, was obtained from the mother liquor.

The compound analyzed as H·*p*-Tos·Orn·OBz·H_3PO_4. Calcd. for $C_{19}H_{27}O_8N_2PS$(474.5): N, 5.91; C, 48.1; H, 5.74; P, 6.53. Found: N, 5.88; C, 47.83; H, 5.74; P, 6.1.

2. δ-*p*-Toluenesulfonyl-L-ornithine Benzyl Ester Hydrochloride.—15.7 g. (0.055 mole) of δ-*p*-toluenesulfonyl-L-ornithine was dissolved in 95 ml. of benzyl alcohol containing 26 g. of polyphosphoric acid. After stirring for 4 hr. at 95° the reaction mixture was cooled to room temperature and one volume of anhydrous ether added. The solution was then saturated with anhydrous hydrogen chloride. Another volume of ether was added and the solution was

(1) Paper I: B. F. Erlanger, H. Sachs and E. Brand, THIS JOURNAL, **76**, 1806 (1954); paper II: B. F. Erlanger, W. V. Curran, N. Kokowsky, *ibid.*, **80**, 1128 (1958); paper III: B. F. Erlanger, W. V. Curran, N. Kokowsky, *ibid.*, **81**, 3051 (1959).

(2) This research is supported by the Office of Naval Research under contract N-onr-266(44).

(3) For an explanation of the abbreviations, see papers I, II and III (ref. 1). Briefly: Z, carbobenzyloxy, $C_6H_5CH_2OCO$; *p*-Tos, *p*-toluenesulfonyl, $C_7H_7SO_2$; Leu, leucyl, $NH(CHC_4H_9)CO$; Val, valyl, $NH(CHC_3H_7)CO$; etc. The configurations of the amino acid residues appear in parentheses after the name of the compound.

(4) B. F. Erlanger and R. M. Hall, THIS JOURNAL, **76**, 5781 (1954).

(5) V. Prelog and P. Wieland, *Helv. Chim. Acta*, **29**, 1128 (1946).

(6) The compounds are numbered to correspond with the numbering in Table I.

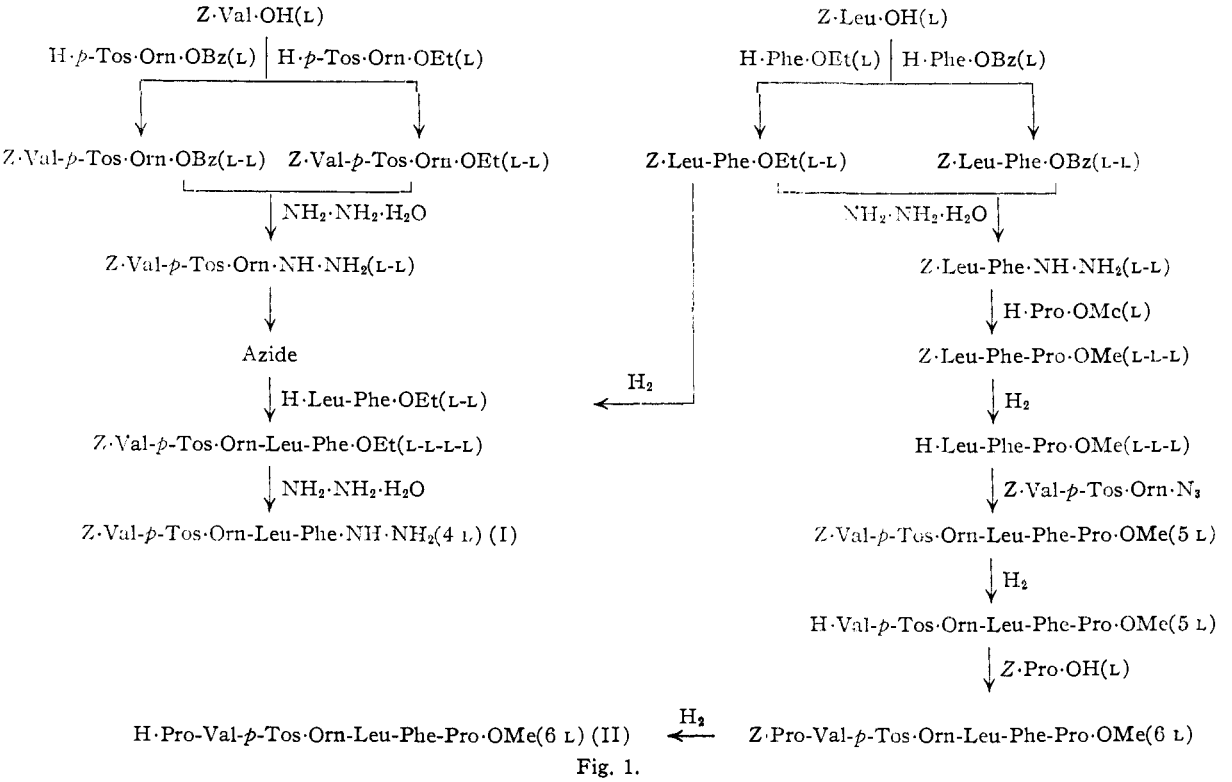

Fig. 1.

retreated with hydrogen chloride. Four volumes of dry ether were added and the compound crystallized slowly over a 48-hr. period at room temperature. After standing in the refrigerator for 24 hr. the product was filtered and washed with dry ether; yield 19.3 g. (85%). Two recrystallizations from methanol–ether gave 12.6 g. (56%), m.p. 125–127°. *Anal.* Calcd. for $C_{19}H_{25}O_4N_2SCl$ (412.9): N, 6.7. Found: N, 6.6.

3. **δ-*p*-Toluenesulfonyl-L-ornithine Ethyl Ester Hydrochloride.**—21.8 g. (0.076 mole) of δ-*p*-toluenesulfonyl-L-ornithine (ref. 1, paper I) was suspended in 600 ml. of absolute ethanol and treated for ten minutes with dry hydrogen chloride in an ice–salt bath. The solution was allowed to stand overnight in the refrigerator and again hydrogen chloride was passed in for ten minutes at ice–salt temperature. The ethanol was then removed *in vacuo* and resulting oil was taken up in 500 ml. of absolute alcohol and retreated twice with hydrogen chloride, each time removing the solvent *in vacuo*. Finally an oil was obtained which could not be crystallized and was used as such.

4. **Z-Leu-Phe·OEt(L-L).**—12.7 g. (0.048 mole) of Z-Leu·OH(L)[7] was dissolved in 50 ml. of dioxane and 11.4 ml. (0.048 mole) of tri-*n*-butylamine. The solution was cooled to 10° and 4.6 ml. (0.048 mole) of ethyl chlorocarbonate added slowly with swirling. After standing 30 minutes at 10°, it was added to a previously cooled solution of 11.0 g. (0.048 mole) of H·Phe·OEt·HCl(L)[8] and 11.4 ml. (0.048 mole) of tri-*n*-butylamine in 50 ml. of dioxane. Carbon dioxide evolution took place immediately upon mixing. The solution was allowed to stand at room temperature for 2 hr., then in the refrigerator overnight. The oily dipeptide derivative obtained by addition of 300 ml. of water was extracted with three 100-ml. portions of ethyl acetate, and the ethyl acetate extract washed with dilute hydrochloric acid, dilute sodium bicarbonate and water then dried over sodium sulfate. After removal of the solvent *in vacuo* the product crystallized as needles. The compound was recrystallized from ethyl acetate–petroleum ether; yield 16.9 g. (80%), m.p. 94–95°, $[\alpha]^{22}D$ −24.0 (1% in methanol).

5. **Z-Leu-Phe·OBz(L-L).**—This compound was prepared by the same procedure described for the corresponding ethyl ester (compound 4) with the exceptions that isobutyl chlorocarbonate was used in the preparation of the mixed anhydride and tetrahydrofuran as the solvent. 18.65 g. (0.072 mole) of Z·Leu·OH(L) and 20.4 g. (0.702 mole) of H·Phe·OBz·HCl(L)[4] gave 24.3 g. (69%) of the carbobenzoxy dipeptide ester. Recrystallization from ethyl acetate–petroleum ether yielded 17.1 g. (49%), m.p. 119–120°. Four g. (60%, total yield), m.p. 115–118° was obtained from the mother liquor; $[\alpha]^{22}D$ −32.7 (1% in methanol).

6. **Z-Leu-Phe·NH·NH$_2$(L-L).**—15.0 g. (0.034 mole) of Z-Leu-Phe·OEt(L-L) (compound 4) was dissolved in 50 ml. of anhydrous methanol and 3.5 ml. (0.072 mole) of hydrazine hydrate was added. The solution was refluxed for an hour on a steam-bath. After standing overnight in the refrigerator, the hydrazide was filtered off, washed with anhydrous methanol and dried *in vacuo*; yield 11.8 g. (81%), m.p. 186–188°, $[\alpha]^{25}D$ −24.1 (1% in glacial acetic acid).

This compound was also prepared in 83% yield from the benzyl ester.

7. **H·Leu-Phe·OEt·HCl(L-L).**—11.0 g. (0.25 mole) of Z-Leu-Phe·OEt(L-L) (compound 4) was dissolved in 150 ml. of methanol containing 12.5 ml. of 2 *N* hydrochloric acid. The solution was hydrogenated, using palladium black as a catalyst, until carbon dioxide evolution ceased. After filtration, the methanol was removed *in vacuo*, yielding a crystalline residue. The product was taken up in methanol and crystallized by the addition of ether; yield 6.75 g. (79%), m.p. 190–192°, $[\alpha]^{25}D$ +10.6 (2% in methanol).

8. **Z-Val-*p*-Tos·Orn·OEt(L-L).**—This compound was prepared by a procedure similar to that described for the preparation of Z-Leu-Phe·OEt(L-L) (compound 4). Isobutyl chlorocarbonate was used in preparing the mixed anhydride with tetrahydrofuran as the solvent. The yield from 22.6 g. (0.090 mole) of Z·Val·OH(L)[9] and 26.75 g. (0.076 mole) of H·*p*-Tos·Orn·OEt·HCl(L) (compound 3) was 28.3 g. (71%), m.p. 116–118°.

9. **Z-Val-*p*-Tos·Orn·OBz(L-L).**—This compound was also prepared through the mixed anhydride procedure described for Z-Leu-Phe·OEt(L-L) (compound 4), using isobutyl chlorocarbonate for the anhydride formation and tetrahydrofuran as solvent. 23.0 g. (0.0557 mole) of H·*p*-Tos·Orn·OBz·HCl(L) (compound 2) and 14.0 g. (0.0557 mole) of Z·Val·OH(L)[9] gave 19.8 g. (58%), m.p. 138–139°, after recrystallizing from ethyl acetate–petroleum ether and 80% methanol, $[\alpha]^{25}D$ −27.1 (1% in methanol).

(7) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(8) E. Fischer and W. Schoeller, *Ann.*, **357**, 1 (1907).

(9) R. L. M. Synge, *Biochem. J.*, **42**, 99 (1948).

TABLE I

No.	Compound	Mol. formula	Mol. wt.	M.p., °C.	Nitrogen, % Calcd.	Nitrogen, % Found	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
4	Z·Leu-Phe·OEt(L-L)	$C_{24}H_{32}O_5N_2$	440.5	94–95	6.4	6.3				
5	Z·Leu-Phe·OBz(L-L)	$C_{30}H_{34}O_5N_2$	502.6	119–120	5.5	5.4				
6	Z·Leu-Phe·NH·NH_2(L-L)	$C_{23}H_{30}O_4N_4$	426.5	185–188	13.1	13.1				
7	H·Leu-Phe·OEt·HCl(L-L)	$C_{17}H_{27}O_3N_2Cl$	342.9	192–193	8.2	8.2				
8	Z·Val-*p*-Tos·Orn·OEt(L-L)	$C_{27}H_{37}O_7N_3S$	547.6	116–118	7.7	7.5				
9	Z·Val-*p*-Tos·Orn·OBz(L-L)	$C_{32}H_{39}O_7N_3S$	610.7	134–135.5	6.9	7.0				
10	Z·Leu-Phe-Pro·OMe(L-L-L)	$C_{29}H_{37}O_6N_3$	523.6	109–110	8.0	7.7				
11	H·Leu-Phe-Pro·OMe·HCl(L-L-L)	$C_{21}H_{32}O_4N_3Cl$	426.0	221–223	9.9	10.1				
12	Z·Val-*p*-Tos·Orn-Leu-Phe·OEt(L-L-L-L)	$C_{42}H_{57}O_9N_5S$	818.0	201–203	8.6	8.7	61.6	62.0	7.0	7.0
13	Z·Val-*p*-Tos·Orn-Leu-Phe·NH·NH_2(L-L-L-L)	$C_{40}H_{55}O_8N_7S$	794.0	228–233.5	12.3	12.2	60.5	60.5	7.0	7.0
14	Z·Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe(L-L-L-L-L)	$C_{46}H_{62}O_{10}N_6S$	891.1	174.5–176	9.4	9.3	62.0	62.2	7.0	6.9
15	H·Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe·HCl (L-L-L-L-L)	$C_{36}H_{57}O_8N_6SCl$	793.4	.....	10.6	10.7	57.6	57.3	7.3	7.0
16	Z·Pro-Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe (L-L-L-L-L-L)	$C_{61}H_{69}O_{11}N_7S$	988.2	175–178	9.9	10.0	62.0	62.0	7.0	7.0
17	H·Pro-Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe·HCl (L-L-L-L-L-L)	$C_{45}H_{64}O_9N_7SCl$	890.5	171–175	11.0	11.0	57.9	57.2	7.2	7.4

The above compound was also prepared in 41% yield, using the H·*p*-Tos·Orn·OBz·H_3PO_4(L) (compound 1).

10. **Z·Leu-Phe-Pro·OMe**(L-L-L).—12.7 g. (0.0298 mole) of Z·Leu-Phe·NH·NH_2(L-L) (compound 6) was dissolved in a solution of 100 ml. of glacial acetic acid, 22 ml. of 5 *N* hydrochloric acid and 330 ml. of water. After cooling to 0°, 2.1 g. (0.03 mole) of sodium nitrite was added and the precipitated azide extracted with cold ethyl acetate. The extract was washed with cold solutions of water, dilute sodium bicarbonate and water and then dried over magnesium sulfate in the cold. To this was added a cold, dry solution of H·Pro·OMe(L) (ref. 1, paper I) previously prepared from 7.4 g. (0.447 mole) of the ester hydrochloride. The reaction mixture was allowed to stand at room temperature overnight and then washed with dilute hydrochloric acid, water, dilute sodium bicarbonate, water and dried over magnesium sulfate. Removal of the ethyl acetate *in vacuo* yielded an oil which was crystallized from ether–petroleum ether; yield 3.1 g., m.p. 109–110°, $[\alpha]^{20}D$ −69.5 (0.5% in methanol). A second recovery of 0.5 g., m.p. 107–108.5°, and a third 2.8 g., m.p. 104–106° (40% total yield), was obtained from the mother liquor. Attempts to obtain additional crystalline material were unsuccessful so the solvents were removed *in vacuo* and the resulting oil hydrogenated as such (see compound 11).

11. **H·Leu-Phe-Pro·OMe·HCl**(L-L-L). **(A) From the Crystalline Z·Leu-Phe-Pro·OMe**(L-L-L).—6.1 g. (0.0117 mole) of the crystalline carbobenzoxy tripeptide ester was dissolved in 100 ml. of methanol containing 7 ml. of 2 *N* hydrochloric acid and hydrogenated over palladium black for 3.5 hr. The catalyst was filtered off and the solvents removed *in vacuo*. The oily ester hydrochloride crystallized from a methanol–ether solution; yield 3.76 g. (76%), m.p. 221–223° (decomposition, $[\alpha]^{21.5}D$ −23.9 (2%i n methanol).

(B) From the Oily Z·Leu-Phe-Pro·OMe(L-L-L).—The carbobenzoxy tripeptide ester was dissolved in 100 ml. of methanol containing 14 ml. of 2 *N* hydrochloric acid and hydrogenated, as described for the crystalline material; yield (after recrystallization from methanol–ether), 2.5 g., m.p. 221–223° (decomposition); mixed m.p. with material prepared from crystalline compound 10, 220–223° dec., $[\alpha]^{22.5}D$ −23.8 (2% in methanol).

12. **Z·Val-*p*-Tos·Orn-Leu-Phe·OEt**(L-L-L-L).—10.45 g. (0.0196 mole) of Z·Val-*p*-Tos·Orn·NH·NH_2(L-L) (ref. 1, paper I) was dissolved in 83 ml. of glacial acetic acid, 41 ml. of 1 *N* hydrochloric acid and 335 ml. of water. After cooling to 0°, it was converted to the azide with 1.37 g. (0.0199 mole) of sodium nitrite; the azide was extracted, washed and dried, as described in the preparation of Z·Leu-Phe-Pro·OMe(L-L-L) (compound 10). A cold, dry ethyl acetate solution of the free ester, prepared from 6.7 g. (0.0196 mole) of H·Leu-Phe·OEt·HCl(L-L), was added to the azide and the solution allowed to stand overnight in the cold. After bringing to room temperature for 4 hr. and then chilling again, the product was filtered off; yield 12 g. (75%), m.p. 198–200°. Recrystallization from aqueous dioxane gave 10.1 g. (64%), m.p. 201–203°, $[\alpha]^{20}D$ −28.6 (1% in methanol).

13. **Z·Val-*p*-Tos·Orn-Leu-Phe·NH·NH_2**(L-L-L-L).—9.0 g. (0.0111 mole) of Z·Val-*p*-Tos·Orn-Leu-Phe·OEt(L-L-L-L) (compound 12) was suspended in 90 ml. of methanol containing 1.62 ml. (0.0324 mole) of hydrazine hydrate. The mixture was refluxed for 18 hr., cooled overnight in the refrigerator, the precipitate collected by filtration and washed with cold methanol; yield 8.0 g. (90%), m.p. 228–233.5°, $[\alpha]^{25.5}D$ −27.6 (2% in glacial acetic acid).

14. **Z·Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe**(L-L-L-L-L).—7.3 g. (0.0136 mole) of Z·Val-*p*-Tos·Orn·NH·NH_2(L-L) (ref. 1, paper I) was dissolved in 70 ml. of glacial acetic acid, 16 ml. of 2 *N* hydrochloric acid and 150 ml. of water. The azide was prepared by addition of 0.95 g. (0.0137 mole) of sodium nitrite to the solution, which had been previously cooled to 0°. After extracting into cold ethyl acetate, the azide was washed and dried, as described in the preparation of Z·Leu-Phe-Pro·OMe(L-L-L) (compound 10). A cold, dry ethyl acetate solution of H·Leu-Phe-Pro·OMe(L-L-L) (compound 11) was prepared from 5.8 g. (0.0136 mole) of the hydrochloride. The two solutions were mixed and allowed to stand in the refrigerator overnight and then overnight at room temperature. The product was collected by filtration, washed with cold ethyl acetate and dried *in vacuo;* yield 5.3 g., m.p. 171.5–173.5°. The filtrate was washed with dilute hydrochloric acid, water, dilute sodium bicarbonate, water and dried over magnesium sulfate. The ethyl acetate was removed *in vacuo* and the residue dissolved in a small volume of hot ethyl acetate. On cooling, crystals appeared; yield 3.3 g. (71% total yield), m.p. 175–177°. The two products were combined and recrystallized from 150 ml. of 70% aqueous ethanol to give 8.2 g. (68%), m.p. 174.5–176°, $[\alpha]^{25}D$ −61.8 (0.5% in methanol).

15. **H·Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe·HCl**(L-L-L-L-L).—8.9 g. (0.01 mole) of Z·Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe(L-L-L-L-L) (compound 14) was suspended in 150 ml. of methanol containing 15 ml. of 1 *N* hydrochloric acid. Palladium black was added and hydrogen passed through the suspension. After 1.5 hr., solution was complete. After cessation of carbon dioxide evolution, hydrogenation was stopped, the catalyst filtered off and the solvents removed *in vacuo*. The residue was redissolved in methanol, which was removed *in vacuo*. This procedure was repeated once again using methanol, then twice with ethanol. The resulting oil could not be crystallized but was converted to a glass-like solid under high vacuum; yield 7.3 g. (93%), $[\alpha]^{24}D$ −60.8 (0.5% in 0.1 *N* hydrochloric acid).

16. **Z·Pro-Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe** (L-L-L-L-L-L).—2.5 g. (10.74 mmoles) of Z·Pro·OH(L)[10] was dissolved in 30 ml. of tetrahydrofuran and 2.45 ml. (10.24 mmoles) of tri-*n*-butylamine. The solution was cooled to −8° and 1.35 ml. (10.24 mmoles) of isobutyl chlorocarbonate added slowly with swirling. The solution was then allowed to stand at 0° for 30 minutes. During this time 7.1 g. of H·Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe·HCl(L-L-L-L-L) (compound 15) was dissolved in 50 ml. of warm water. After cooling to room temperature, 1.5 g. (17.9 mmoles) of sodium bicarbonate, dissolved in 20 ml. of water, was added. The precipitated free ester was extracted with three 50-ml. portions of ethyl acetate which was then washed three times with 30 ml. of water and dried over magnesium sulfate. After replacing the ethyl acetate with tetrahydrofuran and cooling to 0°, the solution was added to the mixed anhydride and stored overnight in the cold. Most of the solvent was

(10) E. Abderhalden and H. Nienberg, *Fermentforschung*, **13**, 573 (1933).

removed *in vacuo* and the reaction mixture poured into five volumes of water. The carbobenzoxy hexapeptide ester was extracted into ethyl acetate, which was washed with dilute hydrochloric acid, water, dilute sodium bicarbonate, water and dried over magnesium sulfate. Removal of the ethyl acetate *in vacuo* left a viscous oil, which was solidified by the addition of anhydrous ether; yield 7.7 g. (87%), softens at 174°, m.p. 175–178°, $[\alpha]^{25}D$ −96.0 (0.5% in methanol).

17. H·Pro-Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe·HCl(L-L-L-L-L-L).—7.5 g. (7.6 mmoles) of Z·Pro-Val-*p*-Tos·Orn-Leu-Phe-Pro·OMe(L-L-L-L-L-L) (compound 16) was dissolved in 150 ml. of methanol containing 11.4 ml. of 1 *N* hydrochloric acid and hydrogenated over palladium black until carbon dioxide evolution ceased. The palladium was removed by filtration and the solvent distilled off *in vacuo*. The residual oil was made anhydrous by repeated additions of ethyl alcohol, followed by removal of the alcohol by distillation *in vacuo*. The product crystallized from methanol–ethyl acetate; yield 4.8 g. (72%), $[\alpha]^{25}D$ −81.0 (0.5% in 0.01 *N* hydrochloric acid).

NEW YORK, N. Y.

[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE, THE WEIZMANN INSTITUTE OF SCIENCE]

The Constituents of *Ecballium elaterium* L. VI. The Functions of Elatericin A[1,2]

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The functions of the potent anti-tumor, naturally occurring compound elatericin A have been investigated. The following groupings were identified: an α,β-unsaturated ketone, an α-hydroxy-keto system easily autoxidizable with acid and alkali, and a third ketonic function probably in a hindered position. Elatericin A formed a diacetate, two acylatable hydroxyl groups being present. The molecule has been oxidized with periodic acid.

The isolation of elatericin A has been described in Part III of this series.[3] It occurs in the juice of the fruit of *Ecballium elaterium* and is obtained together with elatericin B by extraction with ether. The same compound has been isolated by Enslin[4a] from *Cucurbita pepo* and has been called by him Cucurbitacin D. This compound was also found in some other species of the Cucurbitaceae.[4]

Elatericin A, as well as some of the other constituents of this plant, has been found to have anti-tumor activity against Sarcoma 37[5] and Black Sarcoma in mice.[6]

The analyses of elatericin A consistently fitted the formula of $C_{28}H_{42}O_7$. Enslin, Rehm and Rivett[4b] suggested that the compounds of this series should be C_{30}-compounds unless an acetic acid ester group had to be attached to the molecule, the compounds being then of the C_{32}-type. The formula of $C_{30}H_{46}O_7 \cdot 0.5\ H_2O$ was therefore attributed to Cucurbitacin D. In view of recent developments on the structure of these compounds, we have adopted this formula, although it is only tentative and may be revised later.

Conclusions regarding the functional groups in elatericin A have been obtained from its spectroscopic data. The presence of an α,β-unsaturated ketone is indicated by a maximum in the ultraviolet light at 230 mμ (ϵ 10,000) (Fig. 1) and by bands at 1635 and 1689 cm.$^{-1}$ in the infrared. When elatericin A was hydrogenated over palladium, one mole of hydrogen was rapidly absorbed to form a dihydroelatericin A. In this compound the strong peak at 230 mμ had disappeared (Fig. 2) and no other strong absorption was found in ultraviolet. The double bond conjugated to the carbonyl group was therefore reduced. When alkali was added to a methanolic solution of elatericin A, an interesting observation was made. The strong peak at 230 mμ in the ultraviolet spectrum decreased rapidly, disappeared[4b] in three hours, and did not reappear upon acidification of the solution as shown in Fig. 1. By the action of alkali therefore, an irreversible change involving the α,β-unsaturated ketone takes place in the molecule. If the alkaline solution was allowed to stand a longer time it was observed that a new peak developed slowly in the ultraviolet at 310 mμ (ϵ 5,100) reaching its maximum 40 hours later.[4b] Acidification of the solution resulted in a shift of this peak to 268 mμ (ϵ 6,900); Fig. 1. The solution now gave a positive test with ferric chloride for a phenol group, and the infrared spectrum of the new product indicated two new bands at 1660 and 1413 cm.$^{-1}$, which were not seen in elatericin A; moreover a band at 1713 cm.$^{-1}$ originally present in that molecule had disappeared. It is noteworthy, that this change in the molecule occurring during addition of alkali was not reversible, and acidification did not restore the original molecule but shifted the new maximum to a shorter wave length. Compared to the rapid disappearance of the maximum at 230 mμ in the ultraviolet the rate of formation of the new peak at 310 mμ is very slow as shown in Fig. 3. There seems therefore to be no interrelationship between these two processes which must be independent; essentially dihydroelatericin A, whose ultraviolet spectrum did not show any maximum at 230 mμ, behaved in a similar way upon addition of alkali and a maximum gradually appeared at 310 mμ, Fig. 2.

The hypsochromic shift of about 52 mμ combined with an increase in intensity of absorption of about 30%, as well as the new infrared bands, clearly

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(2) In this series of papers are to be considered Part IV, D. Lavie and Y. Shvo, *Proc. Chem. Soc.*, 220 (1958); and Part V, D. Lavie, Y. Shvo and D. Willner, *Chemistry & Industry*, 1361 (1958).

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(6) Unpublished data.