Communications to the Editor

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SYNTHESIS AND ADJUVANT ACTIVITY OF FK-156 ANALOGUES: ACYL DERIVATIVES OF *N*-[N^2-(L-ALANYL-γ-D-GLUTAMYL)-2(L),2'(D)-DIAMINO-1-PIMELOYL]GLYCINE

Satoshi Okada, Hidekazu Takeno, Keiji Hemmi, Yoshihiko Kitaura, and Masashi Hashimoto*

Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd. 2-1-6 Kashima, Yodogawa-ku, Osaka 532, Japan

The hydroxy-bearing acyl analogues of FK-156 (1) were synthesized and their adjuvant activities were examined. The new compounds 2-4 showed significant effects in inducing delayed-type hypersensitivity and antibody production superior to those of 1.

KEYWORDS — microbial metabolite; adjuvant activity; delayed-type hypersensitivity; antibody production

The unique chemical and biological properties of FK-156 (1), a new immunostimulating microbial metabolite,[1] have stimulated us to investigate structural modifications of this compound for enhancing its activity. We described in preceding papers the effect of substituting amino acid residues in analogues of 1.[2] Although in those investigations we also substituted some fatty acids for the *N*-terminal lactoyl moiety of 1, here we address questions on the effects of the hydroxy function of this lactoyl group on the activity of 1. We report the synthesis and biological properties of FK-156 analogues 2-4 with hydroxy-bearing acyl groups, which have adjuvant activities superior to those of 1.

The compounds 2-4 were synthesized starting from the intermediate 5 used to synthesize 1.[3] Carbobenzyloxylation of 5 according to the standard manner gave the di-Z derivative 6,[3b] which in turn was treated with HCl/AcOEt (0°C, 3 h) for removal of the Boc group to give, after neutralization with $NaHCO_3$, hydrazide 7 [mp 144-148°C, $[\alpha]_D^{25}$-23.8°(c 0.5, acetone), Rf 0.82(B)] in 56% yield from 5. Oxidation of the hydrazide group in 7 with *N*-chlorosuccinimide in EtOH-CH_2Cl_2 (1 : 1, -5°C), followed by a spontaneously occurring ethanolysis, provided ethyl ester 8 [mp 125-126°C, $[\alpha]_D^{25}$+7.3°(c 0.6, $CHCl_3$), Rf 0.3(A)] in 70% yield. Treatment of 8 with $SOCl_2/CH_2Cl_2$ in the same way as described previously[4] provided *N*-carboxyanhydride 9 [oil, ν($CHCl_3$) 1850, 1780 cm^{-1}], which without purification was allowed to react with benzyl glycinate (Et_3N/CH_2Cl_2, -20~-10°C) to yield 10 [oil, Rf 0.37(A)] in 84% yield from 8. Condensation of 10 with Boc-L-Ala-D-Glu(OH)OBzl[3,5] *via* the active ester procedure using *N*-hydroxysuccinimide (MeCN, room temperature) gave 11 [mp 95-97°C, $[\alpha]_D^{25}$-14.0°(c 0.2, $CHCl_3$), Rf 0.51(A), 77% yield], which was treated with TFA (room temperature, 30 min) to afford tetrapeptide 12 [mp 131-132°C, $[\alpha]_D^{25}$-12.2°(c 0.3, $CHCl_3$, Rf 0.32(A)].

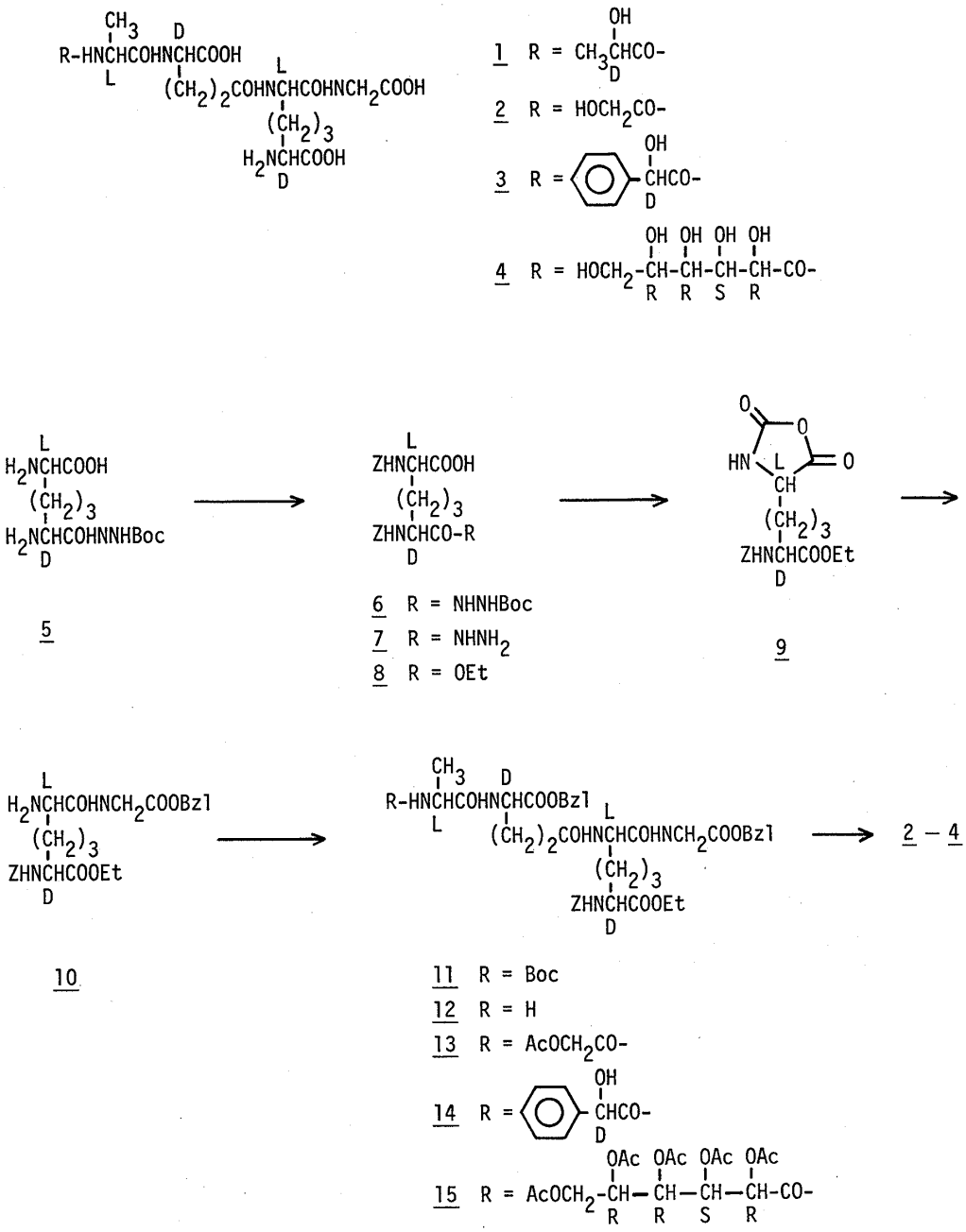

Chart 1

Acylation of 12 with *O*-acetylglycolyl chloride (Et_3N/CH_2Cl_2, 5°C) gave 13 [mp 143-146°C, $[\alpha]_D^{25}$ -14.0° (c 0.4, $CHCl_3$), Rf 0.61(A), 71% yield from 11], which was deprotected by hydrogenolysis (10% Pd-C/MeOH) and subsequent alkaline hydrolysis (dilute NaOH, 0°C) and purified by chromatography on Dia-ion HP-20 (MeOH) to afford 2 [powder; $[\alpha]_D^{25}$ -28.3° (c 0.2, H_2O); Rf 0.22(B); amino acid ratio of the acid hydrolysate: Ala 1.00, Glu 1.01, Gly 0.96, 2,2'-diaminopimelic acid(A_2pm)[5] 0.95; Anal. Calcd for $C_{19}H_{31}N_5O_{11}\cdot 4H_2O$: C, 39.51; H, 6.81; N, 12.13. Found: C, 39.69; H, 6.22;

N, 12.32] in 79% yield.

Compound 3 [powder; $[\alpha]_D^{25}$-43.4°(c 0.3, H_2O); Rf 0.37(B); amino acid ratio of the acid hydrolysate: Ala 1.00, Glu 1.01, Gly 0.96, A_2pm 1.01; Anal. Calcd for $C_{25}H_{35}N_5O_{11}\cdot 1/2H_2O$: C, 50.84; H, 6.14; N, 11.86. Found: C, 50.87; H, 6.45; N, 11.51; 76% yield] was prepared by acylation of 12 with D-mandelic acid using *N*-hydroxysuccinimide/DCC (MeCN, room temperature) to 14 [mp 113-115°C, $[\alpha]_D^{25}$-21.4° (c 0.3, $CHCl_3$), Rf 0.46(A), 70% yield] and successive removal of the protecting groups in a similar manner. A similar sequence from 12 and *O*-pentaacetylgluconic acid *via* 15 [mp 80-81°C, $[\alpha]_D^{25}$-7.8°(c 0.4, $CHCl_3$), Rf 0.58(A), 87% yield] yielded 4 [powder; $[\alpha]_D^{25}$-11.4°(c 0.4, H_2O); Rf 0.17(B); amino acid ratio of the acid hydrolysate; Ala 1.00, Glu 1.02, Gly 1.02, A_2pm 1.07; Anal. Calcd for $C_{23}H_{39}N_5O_{15}\cdot 3H_2O$: C, 40.65; H, 6.53; N, 10.31. Found: C, 40.61; H, 6.22; N, 10.14] in 63% yield.

Table I. Adjuvant Activity on Induction of Skin Reaction and Antibody Production to Ovalbumin in Hartley Guinea Pigs (Male)[a]

Compd.	Dose (μg/site)	Skin reaction[b] (mean ± SE)	Antibody production[c] (mean ± SE)
Controls		0	8.9 ± 0.3
1	1	7.4 ± 1.0*	10.3 ± 0.4*
	10	9.7 ± 0.9*	9.9 ± 0.3*
2	1	10.4 ± 2.4*	11.8 ± 0.5*
	10	14.6 ± 1.1*	12.4 ± 0.5*
3	1	11.5 ± 2.1*	10.8 ± 0.3*
	10	11.1 ± 0.7*	12.3 ± 0.5*
4	1	12.0 ± 0.3*	11.1 ± 0.4*
	10	11.3 ± 1.0*	11.2 ± 0.4*

a) Guinea pigs (five animals in each series) were immunized in both hind footpads with 1 mg of ovalbumin with compounds in Freund's incomplete adjuvant and, after 2 weeks, 5 μg of ovalbumin was given as challenge.
b) After 45 h, skin reactions were estimated by measuring diameters of induration (mm).
c) Hemagglutinin titers were estimated as $\log_2$ of the maximal dilution of sera agglutinating ovalbumin-coated sheep red cells on day 21 after the antigen challenge.
**)* Significantly different from controls ($p < 0.05$).

The adjuvant activity of the new compounds 2-4 was determined by skin reaction (cellular immunity) and antibody production (humoral immunity) in guinea pigs immunized with ovalbumin as shown in comparison with 1 in Table I. All the new compounds exhibited greater activities than 1 in both skin reaction and antibody production assays. It is noticeable that in the former assay 2 and 3 particularly induced a necrosis at the site of dosing, thus proving to be considerably more potent than 1. The enhancement of the adjuvant activity of 1 by replacement of the lactic acid moiety with the other hydroxy-bearing acyl groups is noteworthy, although no quantitative correlations are available at this time concerning the structure-activity relationships among the compounds of this series. The fatty

acid derivatives of FK-156 did not markedly affect the adjuvant potency,[6] which also demonstrates the necessity of the hydroxy groups in eliciting the improved effects.

All the compounds described above also have phagocytic effects in carbon clearance assay and protective effects against bacterial infections. These data will be reported in a forthcoming full paper.

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4) Analytical TLC was performed with Silica gel 60-F_{254} (E. Merck AG) using the following solvent systems: A, $CHCl_3$–MeOH (10 : 1); B, *n*-BuOH–AcOH–H_2O (5: 2 : 3).
5) Abbrebiations used here for amino acids are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature, Eur. J. Biochem., 138, 9 (1984).
6) Details will be reported in due cource.

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