

Compound **2a** was then converted to all-*trans*-1-bromo-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraene (**3a**) by treatment with PBr₃. Without further purification, the bromo compound (**3a**) was allowed to react with sodium *p*-toluenesulfinate dihydrate to give all-*trans*-3,7,11,15-tetramethyl-1-(*p*-tolylsulfonyl)-2,6,10,14-hexadecatetraene (**4a**) in an overall yield of 85%. Compound **4a** was coupled with *trans*,*trans*-1-benzyloxy-8-chloro-7,7-dimethyl-2,6-octadiene (**5**)¹⁰ in the presence of *t*-BuOK to give all-*trans*-1-benzyloxy-3,7,11,15,19,23-hexamethyl-9-(*p*-tolylsulfonyl)-2,6,10,14,18,22-tetracosahexaene (**1b**) (89%) which was subjected to reduction with lithium in ethylamine, giving all-*trans*-3,7,11,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaen-1-ol (**2b**) in 82% yield. The multiprenyl chain elongation of **2b** to all-*trans*-3,7,11,15,19,23,27,31-octamethyl-2,6,10,14,18,22,26,30-triacontaoctaen-1-ol (**2c**) was performed by repeating the above mentioned procedure (**2b** → **3b** → **4b** → **1c** → **2c**). Physicochemical properties of the multiprenyl alcohols (**2**) and their intermediates are listed in Table 1.

TABLE 1. YIELDS AND DATA OF ELEMENTAL ANALYSIS OF MULTIPRENYLALCOHOLS (2)
AND THEIR INTERMEDIATES (1 AND 4)

Compound	n	Yield %	Formula	Found (Calcd)(%)		
				C	H	S
1a	1	91.0	C ₃₄ H ₄₆ O ₃ S	76.24 (76.36)	8.89 8.67	6.19 6.00)
1b	3	88.9	C ₄₄ H ₆₂ O ₃ S	78.66 (78.75)	9.46 9.31	4.69 4.78)
1c	5	86.4	C ₅₄ H ₇₈ O ₃ S	80.46 (80.34)	9.79 9.74	3.98 3.97)
2a	4	95.6	C ₂₀ H ₃₄ O	82.95 (82.69)	12.17 11.80)	
2b	6	82.0	C ₃₀ H ₅₀ O	84.75 (84.44)	12.19 11.81)	
2c^{a)}	8	71.5	C ₄₀ H ₆₆ O	85.56 (85.34)	12.13 11.82)	
4a	4	84.7	C ₂₇ H ₄₀ O ₂ S	75.78 (75.78)	9.73 9.47	7.30 7.48)
4b	6	77.2	C ₃₇ H ₅₆ O ₂ S	78.75 (78.66)	10.02 9.99	5.72 5.68)

a) Wax with low mp.

TABLE 2. YIELDS AND DATA OF ELEMENTAL ANALYSIS OF MULTIPRENYLACETIC ACIDS (7)
AND THEIR INTERMEDIATES (6)

Compound	n	R	Yield %	Formula	Found (Calcd)(%)	
					C	H
6a	4	H	52.0	C ₂₇ H ₄₄ O ₄	74.97 (75.02)	10.35 10.26)
6a'	4		13.4	C ₄₇ H ₇₆ O ₄	79.86 (80.12)	10.89 10.79)
6b	6	H	38.0	C ₃₇ H ₆₀ O ₄	78.48 (78.12)	10.75 10.75)
6b'	6		44.0	C ₆₇ H ₁₀₈ O ₄	82.27 (82.32)	11.20 11.14)
6c^{a)}	8	H	40.7	C ₄₇ H ₇₆ O ₄	80.29 (80.06)	11.05 10.87)
6c' a)	8		16.6	C ₈₇ H ₁₄₀ O ₄	83.80 (83.59)	11.37 11.29)
6d	2	H	54.2	C ₁₇ H ₂₈ O ₄	69.17 (68.89)	9.67 9.52)
6d'	2		27.1	C ₂₇ H ₄₄ O ₄	75.23 (74.95)	10.47 10.25)
7a	4	H	73.2	C ₂₂ H ₃₆ O ₂	79.46 (79.46)	11.20 10.91)
7a'	4		92.9	C ₄₂ H ₆₈ O ₂	83.13 (83.38)	11.52 11.32)
7b^{a)}	6	H	100	C ₃₂ H ₅₂ O ₂	81.74 (81.99)	11.50 11.18)
7b' a)	6		74.6	C ₆₂ H ₁₀₀ O ₂	85.12 (84.86)	11.77 11.49)
7c^{a)}	8	H	82.0	C ₄₂ H ₆₈ O ₂	83.44 (83.38)	11.47 11.32)
7c' a)	8		96.0	C ₈₂ H ₁₃₂ O ₂	85.62 (85.65)	11.79 11.57)
7d	2	H	89.9	C ₁₂ H ₂₀ O ₂	73.29 (73.42)	10.57 10.27)
7d'	2		85.6	C ₂₂ H ₃₆ O ₂	79.46 (79.46)	11.02 10.91)
7e	3	H	— ¹¹⁾	C ₁₇ H ₂₈ O ₂	77.55 (77.22)	10.64 10.68)

a) Wax with low mp.

a) These compounds were reported previously.⁸⁾ b) Optical rotation was determined when mutarotation was completed (25 h).

dicaboximide (HONB)¹²) active ester by dicyclohexylcarbodiimide (DCC) and coupled with 6-*O*-aminoacyl-*N*-acetylmuramyl dipeptide (**8**)⁸) to give multiprenylacetyl derivatives of MDP with an amino acid as a linking unit (**9**) (Scheme 2). In this study, 6-*O*- β -alanyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine (**8a**) as an analog of a natural MDP type and 6-*O*-L-leucyl-*N*-acetylmuramyl- α -aminoisobutyryl-D-isoglutamine (**8b**) as an analog of an artificial MDP type with higher activity were used. In this acylation, the carboxylic acids with higher molecular weight gave the lower yields. Particularly the acylation with **7c'** could not be accomplished in spite of many trials. In addition to the steric effect, the large difference in the polarities of the two reactants (**7c'** and **8b**) may prevent access of each component to the reacting point. Stearoyl (**10**) and retinoyl (**11**) residues were introduced to **8a** in a similar manner for comparison of the biological activity. The resulting products were purified by column chromatography on silica gel with AcOEt-pyridine-AcOH-H₂O (80:10:3:5) as a solvent system. Rechromatography using Sephadex LH-20 with EtOH as an eluent gave the pure products listed in Table 3.

Biology

The adjuvant activity of these synthetic MDP analogs for the induction of delayed-typed hypersensitivity to *N*-acetyl-3-(4-arsenophenylazo)-L-tyrosine (ABA-Tyr) in guinea pigs was assayed by a method described earlier.¹³) The results are shown in Table 4.

All the compounds reported in this paper showed more potent activity than the saturated stearoyl derivative (**10**). Among them, five compounds (**9b**, **9c**, **9b'**, **9d'**, **11**) revealed higher activity than MDP. The high activity of **9e**, in which the carbon number in the side chain is comparable to that of the stearoyl derivative (**10**), showed that the presence of the multiprenyl structure in the carbon chain is favorable for the activity. The activity of **9d'** is higher than **9a**, indicating that the branched structure of the chain

TABLE 4. ADJUVANT ACTIVITY OF MDP DERIVATIVES **9**, **10**, AND **11** IN DELAYED-TYPE HYPERSENSITIVITY TO ABA-Tyr (100 μ g) IN GUINEA PIGS

Compound ^{a)}	Skin reaction (mm $\pm$ s.e.)	
	24 h	48 h
9d	22.8 $\pm$ 0.6	23.5 $\pm$ 1.7
9e	25.0 $\pm$ 1.3	26.3 $\pm$ 1.6
9a	22.1 $\pm$ 1.2	24.1 $\pm$ 1.4
9b	24.3 $\pm$ 0.7	28.9 $\pm$ 1.3
9c	26.4 $\pm$ 1.2	26.6 $\pm$ 1.3
9d'	25.8 $\pm$ 0.9	29.8 $\pm$ 2.0
9a'	24.0 $\pm$ 1.6	26.1 $\pm$ 1.3
9b'	26.1 $\pm$ 1.4	30.9 $\pm$ 2.6
10	21.1 $\pm$ 1.4	23.1 $\pm$ 2.0
11	24.1 $\pm$ 0.9	28.1 $\pm$ 1.4
MDP	23.9 $\pm$ 1.1	26.9 $\pm$ 1.2
Control (ABA-Tyr + FIA) ^{b)}	0	0

a) Dose: 100 μ g. b) FIA: Freund's incomplete adjuvant.

is also favorable. The remarkable tendency for the compounds with larger side chains (**9b**, **9c**, and **9b'**) to possess increased activity is interesting although there was no clear-cut relation between the chain length and the activity. The high activity of retinoyl derivative (**11**), in spite of its small carbon number in the chain, suggested that there is some additional immunological function caused by retinoyl moiety as in the case of quinonyl derivatives⁹) of MDP.

These results show that the introduction of lipophilic unsaturated carboxylic acids leads to the potentiation of the immunological activity of MDP, thus providing another approach to the development of a new family of immunologically active compounds.

Experimental

Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter. NMR spectra were obtained on Varian EM-360 and EM-390 spectrometers. All chemicals and solvent were reagent grade and used without further purification. The reactions were monitored on TLC with Merck F₂₅₄ silica-gel plates. Evaporation was carried out in a rotary evaporator under reduced pressure at temperatures below 45 °C.

All-trans-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraen-1-ol (**2a**).

Li (10.9 g, 2 g atom) was dissolved in EtNH₂ (300 ml) at -60 °C under N₂. After the solution became blue, a solution of all-*trans*-1-benzyloxy-3,7,11,15-tetramethyl-9-*p*-tolylsulfonyl-2,6,10,14-hexadecatetraene (**1a**) (21 g, 393 mmol) in anhydrous THF (40 ml) was added dropwise for 30 min. The mixture was stirred for 15 min at -60 °C, while blue color was kept. Isoprene (5 ml) and MeOH (100 ml) were carefully added to quench the excess Li. After the careful addition of water (500 ml), the organic solvents were evaporated. The residual aqueous solution was extracted with diisopropyl ether (150 ml $\times$ 3). The organic layer combined was washed with water, and dried over Na₂SO₄. The solvent was evaporated and the resulting residue was purified by column chromatography (7 $\times$ 15 cm) on SiO₂ using hexane-diisopropyl ether (5:3, and then 1:2) as solvent: 10.9 g (95.6%) (Table 1). NMR (CDCl₃): 1.62 (9H, s), 1.70 (6H, s), 1.91–2.18 (12H, m), 4.15 (2H, d), 5.0–5.2 (3H, m), 5.43 (1H, t).

Other Multiprenyl Alcohols (**2b** and **2c**) were prepared from **1b** and **1c** in a similar manner.

All-trans-3,7,11,15-tetramethyl-1-(p-tolylsulfonyl)-2,6,10,14-hexadecatetraene (**4a**). To a solution of **2a** (14.4 g, 49.6 mmol) in absolute THF (70 ml) was added a solution of PBr₃ (5.6 g, 20.7 mmol) in THF (70 ml) dropwise at -7–-10 °C. Then the mixture was stirred at the same temperature for 15 min. The solvent was evaporated and the residue was dissolved in hexane-diisopropyl ether (1:1, 200 ml). The solution was successively washed with 5% NaHCO₃ and water, and then dried over Na₂SO₄. The solvent was evaporated to give all-*trans*-1-bromo-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraene (**3a**). Without further purification, **3a** was dissolved in DMF (150 ml), and sodium *p*-toluenesulfinate tetrahydrate (24.8 g, 99.2 mmol) was added. After stirring for 1 h at room temperature, the mixture was diluted with hexane-diisopropyl ether (1:1, 400 ml), washed with water, and then dried over Na₂SO₄. After evaporation of the solvents, the resulting residue was purified by silica gel chromatography (6 $\times$ 25 cm) using hexane-diisopropyl ether (2:1) as solvent: 18.0 g (84.7%) (Table 1). NMR (CDCl₃): 1.36 (3H, d), 1.61 (9H), 1.68

(3H, s), 1.98—2.25 (12H, m), 2.44 (3H, s), 3.78 (2H, d), 5.10 (3H, m), 5.18 (1H, t), 7.31 and 7.74 (4H).

Compound **4b** was prepared from **2b** in a similar manner.

All-trans-1-benzyloxy-3,7,11,15,19,23-hexamethyl-9-(p-tolylsulfonyl)-2,6,10,14,18,22-tetracosahexaene (1b). To a solution of **4a** (17.7 g, 41.3 mmol) and *trans,trans*-1-benzyloxy-8-chloro-3,7-dimethyl-2,6-octadiene (**5**) (13.1 g, 47 mmol) in absolute THF (100 ml)—DMF (12 ml) was added *t*-BuOK (6.96 g, 62 mmol) at -20°C . After stirring for 20 min at the same temperature, the mixture was diluted with hexane-diisopropyl ether (1:1, 400 ml), and washed with 5% phosphoric acid (400 ml) and water, and then dried over Na_2SO_4 . The solvent was evaporated, and the residue was chromatographed on a silica-gel column (5.5 $\times$ 20 cm) with hexane-diisopropyl ether (1:1) as an eluent: 24.6 g (88.9%) (Table 1). NMR (CDCl_3): 1.25 (3H, d), 1.55 (3H, s), 1.62 (12H, s), 1.70 (3H, s), 1.85—2.16 (16H, m), 2.44 (3H, s), 2.88 (2H, dd), 3.80 (1H, dd), 4.01 (2H, d), 4.50 (2H, s), 4.90 (1H, d), 4.95—5.24 (4H, m), 5.37 (1H, t), 7.30 and 7.71 (4H, m), 7.33 (5H, s).

Compound **1c** was prepared from **4b** and **5** in a similar manner.

Ethyl 2-Ethoxycarbonyl-all-trans-5,9,13,17,21,25-hexamethyl-4,8,12,16,20,24-hexacosahexaenolate (6b) and *Ethyl 2-(All-trans-3,7,11,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaenyl)-2-ethoxycarbonyl-all-trans-5,9,13,17,21,25-hexamethyl-4,8,12,16,20,24-hexacosahexaenolate (6b')*. *All-trans-3,7,11,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaen-1-ol (2b)* (2.13 g, 5 mmol) was converted to the corresponding bromo-compound (**3b**) in a manner similar to that described in the synthesis of **4a**. To a solution of diethyl malonate (800 mg, 5 mmol) in absolute DMF (10 ml) NaH (240 mg, 5 mmol; 50% suspension in mineral oil) was added under N_2 with stirring. After stirring for 20 min at room temperature, the solution was cooled to -10°C . A solution of **3b** in absolute THF (10 ml) was added dropwise at -10 — -6°C . After 30 min, the mixture was allowed to react at room temperature and stirring was continued for an additional 2 h. Water (100 ml) was carefully added and the solution was extracted with diisopropyl ether (100 ml). The organic layer was washed with water and dried over Na_2SO_4 . The solvent was evaporated and the resulting residue was chromatographed on a silica-gel column (4.2 $\times$ 15 cm) with hexane-diisopropyl ether (15:1) to give pure **6b** and **6b'**: 1.09 g (38.0%) and 1.08 g (44.0%) (Table 2). NMR (CDCl_3) for **6b**: 1.27 (6H, t), 1.64 (21H, s), 1.88—2.19 (20H, m), 2.57 (4H, dd), 3.33 (1H, t), 4.17 (4H, q), 4.92—5.62 (6H, m).

Other Esters (6a, 6a', 6c, 6c', 6d, and 6d') were prepared from the appropriate alcohols and diethyl malonate in a similar manner. Geraniol for the preparation of **6d** and **6d'** was purchased from Wako Pure Chemical Industries, LTD, Osaka.

2-(All-trans-3,7,11,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexenyl)-all-trans-5,9,13,17,21,25-hexamethyl-4,8,12,16,20,24-hexacosahexaenoic Acid (7b'). A solution of **6b'** (1.08 g, 1.1 mmol) in 1 M KOH—MeOH—THF (2:5:1, 22 ml) was heated under reflux for 100 h. After cooling to room temperature, 0.5 M HCl (50 ml) was added and the solution was extracted with AcOEt (40 ml). The organic layer was washed with water, dried over Na_2SO_4 , and then evaporated. The residue (dicarboxylic acid) was dissolved in DMSO (5 ml) and the solution was heated at 150°C for 20 min, during which time the evolution of CO_2 was completed. After cooling to room temperature, the mixture was diluted with H_2O (30 ml) and extracted with AcOEt—diisopropyl ether (1:1, 50 ml). The organic layer was washed

with water, dried over Na_2SO_4 , and evaporated. The resulting residue was chromatographed on a silica-gel column (3.8 $\times$ 6 cm) with hexane-diisopropyl ether (4:1): 720 mg (74.6%). (Table 2). NMR (CDCl_3): 1.61 (42H, s), 1.95—2.45 (45H, m), 4.95—5.21 (12H, m).

Other Multiprenylacetic Acids (7a, 7a', 7b, 7c, 7c', 7d, and 7d') were prepared from the corresponding esters in a similar manner.

N-Acetyl-6-O-[[2-(all-trans-3,7,11,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaenyl)-all-trans-5,9,13,17,21,25-hexamethyl-4,8,12,16,20,24-hexacosahexaenoyl]-L-leucyl]muramyl- α -aminoisobutyryl-D-isoglutamine (9b'). To a solution of **7b'** (87.7 mg, 0.1 mmol) and HONB (21.6 mg, 0.12 mmol) in AcOEt—acetonitrile (1:1, 2 ml) was added DCC (24.7 mg, 0.12 mmol) at 0°C . The mixture was stirred at 0°C for 1 h and then at room temperature for 3 h. After filtration of precipitates, the solvent was evaporated. The resulting active ester, *N*-acetyl-6-O-(L-leucyl)muramyl- α -aminoisobutyryl-D-isoglutamine (**8**)⁸ (62 mg, 0.1 mmol) and NEM (26 μl) were dissolved in DMF (1 ml), and the mixture was stirred at 60°C for 70 h. After evaporation of the solvent, the residue was purified by chromatography on a column of silica gel (2 $\times$ 12 cm) with AcOEt—pyridine—AcOH—water (80:10:3:5), followed by rechromatography on a column of Sephadex LH-20 (1.5 $\times$ 90 cm) with EtOH as an eluent: 20 mg (13.5%) (Table 3).

Other MDP Derivatives with Acylated Amino Acid (9) listed in Table 3 were prepared from the appropriate carboxylic acids and MDP derivatives with an amino acid in a similar manner.

Biological Assays. Determination of the adjuvant activity on induction of delayed-type hypersensitivity was carried out according to the method described earlier.¹³⁾

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