Communications to the Editor

[Chem. Pharm. Bull. 27(12)3193—3196(1979)]

UDC 547.466.1'567.04.09 : 615.277.3.011.5.015.44.076.9

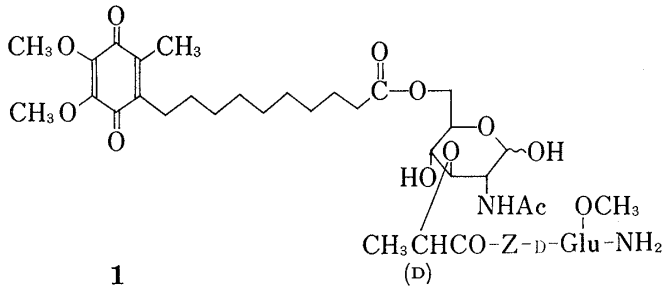
Novel Quinonyl Derivatives of Muramyl Dipeptide Possessing Potent Antitumor Activity[1,2]

Three novel quinonyl muramyldipeptides, 2-{2-acetamido-2-deoxy-6-O-[10-(2,3-dimethoxy-5-methyl-1,4-benzoquinon-6-yl)decanoyl]-D-glucopyranos-3-O-yl}-D-propionyl-L-valyl-D-isoglutamine methyl ester (**1a**) and its Ser and Thr analogues (**1b** and **1c**), were synthesized and their antitumor effects on the suppression of tumor growth in syngeneic mice were assayed. Among these compounds, **1a** showed the most potent antitumor activity.

Keywords——quinonyl acid; muramyl dipeptide; Freund's complete adjuvant; antitumor activity; tumor immunity

Recent findings indicated that N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) is the minimal structural requirement responsible for the activity of Freund's complete adjuvant.[3] This stimulated an extensive search toward elucidating the mechanisms of adjuvant action and developing a class of organic compounds useful for clinical applications.[4] With the purpose of developing potent antitumor immunotherapeutic agents, several investigators have been active in this field. However, MDP and its analogues displayed no antitumor activity in any experimental tumor systems,[5] and modifications of the MDP molecule have been widely undertaken. As a consequence, the α-branched long chain fatty acid derivatives of MDP were found to have distinct antitumor activity,[6,7] suggesting the requirement of a lipophilic nature for the activity.

On this premise, we tried to introduce an immunologically active molecule with a highly lipophilic nature to the MDP molecule with the thought that this might provide another



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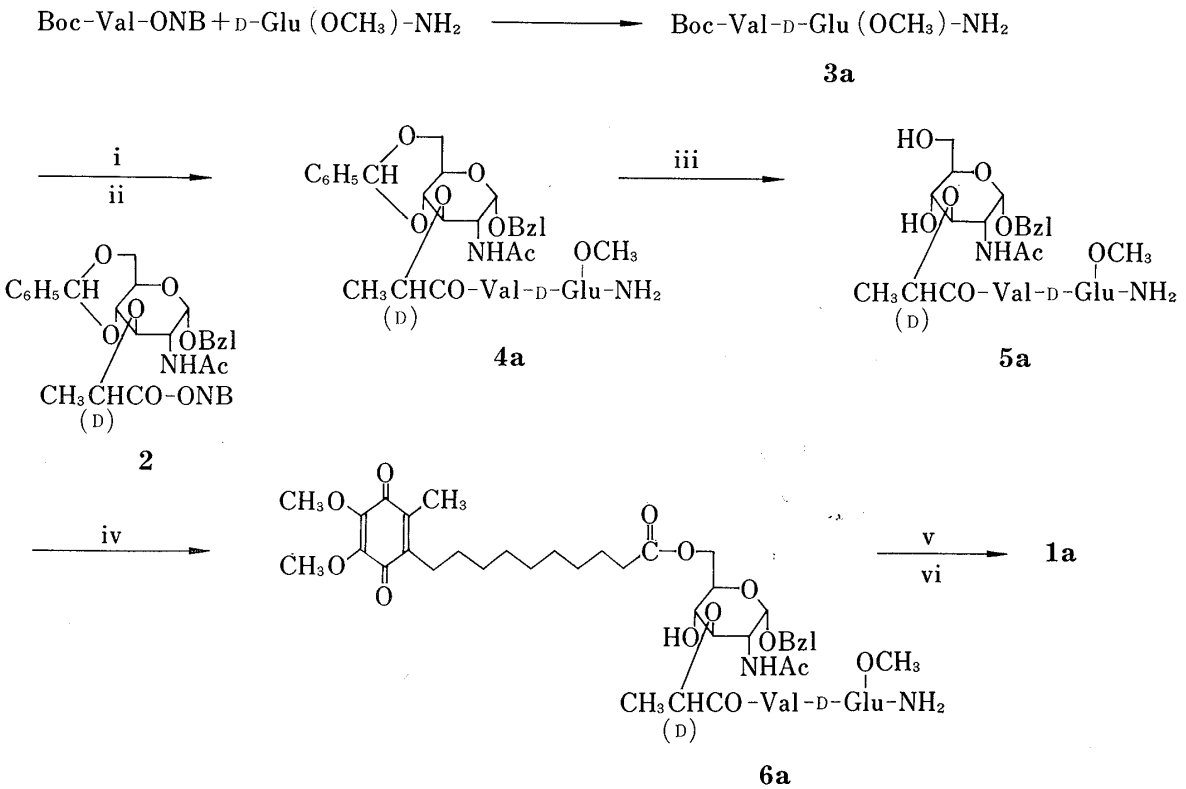
1a: Z=L-Val
1b: Z=L-Ser
1c: Z=L-Thr

1) Presented in part at the National Meeting of the Agricultural Chemical Society of Japan, Tokyo, Apr. 1979, and the 38th Annual Meeting of the Japanese Cancer Association, Tokyo, Sept. 1979.
2) Abbreviations: HONB, N-hydroxy-5-norbornene-2,3-dicarboximide; HOBt, 1-hydroxybenzotriazole; DCC, dicyclohexylcarbodiimide; NEM, N-ethylmorpholine; TEA, triethylamine; DMF, N,N-dimethylformamide.
3) F. Ellouz, A. Adam, R. Ciorbaru, and E. Lederer, *Biochem. Biophys. Res. Commun.*, **59**, 1317 (1974); S. Kotani, Y. Watanabe, F. Kinoshita, T. Shimono, I. Morisaki, T. Shiba, S. Kusumoto, Y. Tarumi, and K. Ikenaka, *Biken J.*, **18**, 105 (1975).
4) For example, see L. Chedid, *Biochem. Pharmacol.*, **27**, 2183 (1978); for a more comprehensive review, see L. Chedid, F. Audibert, and A.C. Johnson, *Prog. Allergy*, **25**, 63 (Karger, Basel, 1978).
5) I. Azuma, K. Sugimura, T. Taniyama, M. Yamawaki, Y. Yamamura, S. Kusumoto, S. Okada, and T. Shiba, *Infect. Immun.*, **14**, 18 (1976).
6) Y. Yamamura, I. Azuma, K. Sugimura, M. Yamawaki, M. Uemiya, S. Kusumoto, S. Okada, and T. Shiba, *Proc. Jpn. Acad.*, **53**, 63 (1977); T. Shiba, S. Okada, S. Kusumoto, I. Azuma, and Y. Yamamura, *Bull. Chem. Soc. Jpn.*, **51**, 3307 (1978).
7) S. Kusumoto, M. Inage, T. Shiba, I. Azuma, and Y. Yamamura, *Tetrahedron Lett.*, **1978**, 4899.

efficient approach to a molecule with more potent antitumor activity. This communication briefly describes the synthesis and antitumor effects of quinonyl MDP (**1**).

10-(2,3-Dimethoxy-5-methyl-1,4-benzoquinon-6-yl)decanoic acid[8] (QS-10) is a highly lipophilic metabolite analogue of ubiquinones (UQ) and replaces some biological characteristics[9] revealed by UQ including enhancement of humoral immune responses.[10] Induction of delayed-type hypersensitivity of QS-10 has also been demonstrated.[11] Thus, our design rationale involved introduction of QS-10 into the MDP molecule through ester linkage, employing the reactions shown in Scheme 1. In this approach, the HONB active ester[12] of Boc–Val and protected muramic acid (**2**) were prepared by the DCC method and separated as crystals.



Step i, TFA; ii, TEA; iii, 75% acetic acid; iv, QS-10-ONp/HOBt/NEM or QS-10/DCC; v H_2/Pd black; vi, $FeCl_3$.

Scheme 1

Dipeptide **3a** was obtained by coupling Boc–Val–ONB with D-Glu–(OCH_3)–NH_2[13] in CH_3CN in 84% yield: mp 117—119°; $[\alpha]_D^{23}$ +8.6° (c=0.5, DMF). Removal of the protecting group of **3a** (TFA for 30 min at room temperature), followed by condensation with **2** afforded the protected muramyl dipeptide **4a** in 83% yield: mp 242° (dec.); $[\alpha]_D^{22}$ +86.1° (c=0.5, DMF).

8) H. Morimoto, I. Imada, M. Watanabe, and M. Kawada, Ger. Patent 2519730 (1975) [*C.A.*, **84**, 58928*j* (1976)].
9) For a review, see I. Imada, M. Watanabe, and H. Morimoto, *Vitamin*, **52**, 493 (1978).
10) I. Azuma, K. Sugimura, Y. Yamamura, R. Bando, M. Watanabe, I. Imada, and H. Morimoto, *Internat. J. Vit. Nutr. Res.*, **48**, 2554 (1978).
11) I. Azuma and I. Imada, unpublished results.
12) M. Fujino, S. Kobayashi, M. Obayashi, T. Fukuda, S. Shinagawa, and O. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **22**, 1857 (1974).
13) D-Glu(OCH_3)–NH_2 was prepared according to the method described for D-Glu(OBzl)–NH_2 by S. Kusumoto *et al.* with modifications; S. Kusumoto, Y. Tarumi, K. Ikenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, **49**, 553 (1976).

Selective deprotection to remove the benzylidene moiety from **4a** was effected by heating **4a** in 75% acetic acid for 20 min on a boiling water bath in 91% yield. Compound **5a** thus obtained [mp 242°; $[\alpha]_D^{23}$ +111.3° (c=0.5, DMF)] was then condensed with the p-nitrophenyl ester of QS-10 (QS-10-ONp, 2 equiv.) in the presence of HOBt and NEM (4 equiv. each) in DMF for 2 days at room temperature[14] to give **6a** in 76% yield: $[\alpha]_D^{21}$ +70.2° (c=0.5, EtOH). An alternate synthesis of intermediate **6a** involved the coupling of **5a** and QS-10 (1 equiv.) with DCC (1.5 equiv.) in a mixture of pyridine and DMF in 70% yield. The conversion of **6a** into **1a** was effected by catalytic hydrogenation in MeOH with palladium black as a catalyst, followed by $FeCl_3$ oxidation in aqueous MeOH. The desired compound **1a** was purified by crystallization from MeOH–ether as orange crystals. The overall yield was 59% based on **6a**.

The corresponding L-Ser (**1b**) and L-Thr (**1c**) analogues were synthesized in a similar manner with Boc–L–Ser(OBzl) and Boc–L–Thr(OBzl) as the starting material, respectively. Analytical data and the physicochemical properties of the final products were summarized in Table I together with those of **1a**.

Table I. Analytical Data and Physicochemical Properties

Compd.	mp[a] (°C)	$[\alpha]_D$, c=0.5 (solvent, temp.)	Formula[b]
1a	188—189	+35.2° (EtOH, 21)	$C_{41}H_{64}N_4O_{16}$
1b	149—150	+39.2° (MeOH, 22)	$C_{39}H_{60}N_4O_{17}$
1c	147—148	+37.2° (MeOH, 21)	$C_{40}H_{62}N_4O_{17}$

a) Melting points were determined in an open capillary and not corrected.
b) All compounds were analyzed for C, H, N. Analytical results for those elements agreed with calculated values within ±0.4%.

Table II. Antitumor Activity of Quinonyl MDP against the Meth-A System

Compd.	Dose (μg)	No. of mice tested	Tumor free mice/mice surving on day 49
1a	100	10	10/10
1b	100	10	3/10
1c	100	10	6/10
MDP	100	10	0/10
Control	—	10	0/10

Antitumor effects of the novel quinonyl MDP derivatives (**1**) on the suppression of tumor growth in mice were evaluated according to the method described earlier.[15] When a mixture of the tumor cells (fibrosarcoma Meth A, 10^5 cells) and **1** suspended in phosphate-buffered saline was inoculated intradermally into syngeneic BALB/c female mice, tumor growth at an inoculated site was suppressed indicating that all of these derivatives exhibited potent antitumor activity (Table II). Moreover, specific and systemic tumor immunity were also induced in mice in which tumor growth was suppressed. Among the compounds tested, **1a** appeared to be of particular interest because of its high efficacy. MDP was shown to be inactive by the same experiment.

14) Acylation was conducted according to a method described earlier for the synthesis of depsipeptides with some modifications; Y.S. Klausner and M. Chorev, *Chem. Commun.*, **1974**, 973.
15) Y. Yamamura, I. Azuma, T. Taniyama, E. Ribi, and B. Zbar, *Gann*, **65**, 179 (1974).

Acknowledgement We wish to thank Drs. E. Ohmura, K. Morita and M. Nishikawa for their encouragement during the course of this work. Thanks are also extended to Dr. M. Watanabe for technical assistance and Professor T. Shiba for discussion.

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Received October 3, 1979

[Chem. Pharm. Bull. 27(12)3196—3198(1979)]

UDC 615.356 : 577.161.011.5.547.92.04

Synthesis of 24,24-Difluoro-1α,25-dihydroxyvitamin D_3

24,24-Difluoro-1α,25-dihydroxyvitamin D_3 (**1**) has been synthesized from 24,24-difluoro-5β-cholestane-3α,25-diol (**2**) as an antimetabolic analogue of 1α,25-dihydroxyvitamin D_3 to study the role of the 24-hydroxylation in the metabolism of vitamin D_3.

Keywords——antimetabolic analogue of 1α,25-dihydroxyvitamin D_3; 24,24-difluoro-5β-cholestane-3α,25-diol; role of 24-hydroxylation of vitamin D_3 in the metabolism; biologically potent vitamin D analogue; NMR spectra

Vitamin D_3 must be metabolically hydroxylated first in the liver at the 25-position and subsequently in the kidney at the 1α-position to afford 1α,25-dihydroxyvitamin D_3 before it can function.[1] Another important hydroxylation occurs at the 24-position under the conditions whereby the 1α-hydroxylation is suppressed[2] and leads 25-hydroxyvitamin D_3 to 24,25-dihydroxyvitamin D_3[3] and 1α,25-dihydroxyvitamin D_3 to 1α,24,25-trihydroxyvitamin D_3.[4] Although the 24-hydroxylated metabolites have significant activity[5] and 24,25-dihydroxyvitamin D_3 is one of the major metabolites, the role of these metabolites has not been clearly understood.

To study the biological importance of 24-hydroxylation in the function of vitamin D, we undertook the synthesis of the analogues of 25-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3 blocked at the 24-position with fluorine atoms and in the previous paper,[6] reported

1) H.F. DeLuca and H.K. Schnoes, *Ann. Rev. Biochem.*, **45**, 631 (1976).
2) H.F. DeLuca, *Life Sciences*, **17**, 1351 (1975).
3) M.F. Holick, H.K. Schnoes, H.F. DeLuca, R.W. Gray, I.T. Boyle, and T. Suda, *Biochemistry*, **11**,4251 (1972); T. Suda, H.F. DeLuca, H.K. Schnoes, G. Ponchon, Y. Tanaka, and M.F. Holick, *Biochemistry*, **9**, 2917 (1970).
4) Y. Tanaka, H. Frank, H.F. DeLuca, N. Koizumi, and N. Ikekawa, *Biochemistry*, **14**, 3293 (1975).
5) M.F. Holick, L.A. Baxter, P.K. Schraugrogel, T.E. Tavela, and H.F. DeLuca, *J. Biol. Chem.*, **251**, 397 (1976).
6) S. Yamada, M. Ohmori, and H. Takayama, *Tetrahedron Lett.*, **1979**, 1859; This compound was also synthesized by an another route, Y. Kobayashi, T. Taguchi, T. Terada, J. Oshida, M. Morisaki, and N. Ikekawa, *Tetrahedron Lett.*, **1979**, 2023.