[Chem. Pharm. Bull.]
34(8)3440-3444(1986)

Enzymatic Synthesis of 2,3-*O*-Isopropylidene-*sn*-glycerol, a Chiral Building Block for Platelet-Activating Factor

HIROSHI SUEMUNE,^a YUKAKO MIZUHARA,^a HIROYUKI AKITA,^b
and KIYOSHI SAKAI*,^a

Faculty of Pharmaceutical Sciences, Kyushu University,^a 3-1-1, Maidashi, Higashi-ku, Fukuoka 812, Japan and The Institute of Physical and Chemical Research (RIKEN),^b Wako-shi, Saitama 351-01, Japan

(Received February 13, 1986)

An enzymatic synthesis of 2,3-*O*-isopropylidene-*sn*-glycerol (**10**), the synthetic key intermediate for platelet-activating factor, was achieved. Several 1,3-di-*O*-acyl-2-*O*-benzylglycerols (**5a—d**) were synthesized from dihydroxyacetone dimer (**2**), and subjected to enzyme-catalyzed asymmetric hydrolysis. The optical purities of the mono-hydrolyzed products (**6**) were determined from the 400 MHz proton nuclear magnetic resonance spectra after conversion of **6** to the esters of (–)- α -methoxy- α -trifluoromethylphenylacetic acid. Upon hydrogenolysis of the benzyl ether, followed by protection of diol and hydrolysis of the acetate, (–)-**6a** afforded **10**.

Keywords—platelet-activating factor; asymmetric hydrolysis; enzymic hydrolysis; 2,3-*O*-isopropylidene-*sn*-glycerol

Platelet-activating factor (PAF, **1**) was first detected as a component capable of rabbit platelet stimulation. The structure of this first bioactive phospholipid has been clarified by Hanahan *et al.*¹⁾ as 1-*O*-hexadecyl (or octadecyl)-2-acetyl-*sn*-glycero-3-phosphorylcholine. PAF is able to provoke platelet and neutrophil activation, hypotension and bronchoconstriction.^{2–4)}

As a part of our attempts to develop a simple synthetic route to PAF, we previously reported an asymmetric synthesis⁵⁾ by (*S*)-BINAL-H⁶⁾ reduction of octadecyloxymethyl (*E*)-2-cyclohexylvinyl ketone. The present paper describes the asymmetric synthesis of 2,3-*O*-isopropylidene-*sn*-glycerol (**10**),⁷⁾ a building block for PAF, by means of a sequence involving an enzymic hydrolysis of *meso*-diacyloxy esters.⁸⁾

The retro synthesis of PAF is shown in Chart 1. This sequence starts with the synthesis of 1,3-di-*O*-acyl-2-*O*-benzylglycerol (**5a—d**), followed by enzymic hydrolysis. 1,3-Di-*O*-acetyl-2-

O-benzylglycerol (**5a**) could be synthesized from commercially available dihydroxyacetone dimer (**2**) by a method using $\text{Zn}(\text{BH}_4)_2$ reduction, as shown in Chart 2. Acetylation of **2** with acetic anhydride in pyridine proceeded smoothly in quantitative yield to afford the keto-diacetate (**3**). Reduction of the ketone function in **3** with NaBH_4 afforded a complex mixture, but reduction under neutral conditions with $\text{Zn}(\text{BH}_4)_2$ in ether afforded 1,3-di-*O*-acetyl-glycerol (**4**) in 81% yield. Reaction of **4** with benzyl chloride and NaH in dimethyl sulfoxide (DMSO) yielded a mixture of **5a** and partially hydrolyzed 1-*O*-acetyl-2-*O*-benzylglycerol (($\pm$)-**6a**) in 34% and 19% yields, respectively.

The analogous diacyl compounds (**5b—d**) were prepared from the diol (**7**),⁹⁾ which was obtained by the hydrolysis of **5a** and/or ($\pm$)-**6a** with K_2CO_3 in MeOH. Acylations of **7** with propionic anhydride, butyric anhydride or chloroacetic anhydride in pyridine afforded the acylates (**5b—d**) in good yields. Diacylation of **7** with acyl chlorides instead of the acid anhydrides afforded complex mixtures.

Chart 2

Enantioselective hydrolysis of the *meso*-compounds (**5a—d**) was studied with commercially available enzymes or microorganisms such as pig liver esterase (PLE), pig pancreatic lipase (PPL), lipase from *Candida cylindracea* and *Saccharomyces cerevisiae* (baker's yeast). The chemical yields, specific rotations and optical yields of the hydrolyzed products are shown in Table I. Optical yields of the mono-hydrolyzed products were estimated from the relative intensities of the two methoxy functions in the 400 MHz proton nuclear magnetic resonance (^1H -NMR) spectra after conversion into (–)- α -methoxy- α -trifluoromethylphenylacetic acid (MTPA) esters by treatment with (–)-MTPA chloride. In the case of **5a** as a substrate, PLE and PPL (Table I, runs 1 and 2) gave the monoacetate (**6a**) in moderate yields, and the signs of specific rotation of each product were different. In the cases of other substrates (**5b—d**) with PLE as the enzyme, the diol (**7**) was obtained as a major product, and the monoacetates (**6b—d**) were obtained in low yields. Among the tested esters, the highest optical yields were approximately 40% ee (runs 1 and 2).

The absolute configuration of (–)-**6a** was determined by conversion into the synthetic intermediate (**10**) of natural PAF as shown in Chart 3. Catalytic hydrogenolysis of (–)-**6a** ($[\alpha]_{\text{D}}^{17} - 6.4^\circ$) with $\text{H}_2/5\%$ Pd–C afforded 1-*O*-acetyl-*sn*-glycerol (**8**) in 80% yield. By treatment

TABLE I. Enzymic Hydrolysis of Diacylates (5a—d)

Run	Substrate (R)		Enzyme	Chemical yield		6	
				(% ^a)		[α] _D (c, CHCl ₃)	Optical yield (% ee)
6	7						
1	5a	CH ₃	PLE	54	14	−6.4° (c=6.30)	39.0
2	5a	CH ₃	PPL	79	3	+6.4° (c=3.23)	40.7
3	5a	CH ₃	Lipase from <i>Candida cylindracea</i>	47	2	+0.2° (c=5.77)	2.0
4	5a	CH ₃	Baker's yeast	29	1	−5.2° (c=3.18)	32.7
5	5b	CH ₂ CH ₃	PLE	9	61	−6.0° (c=1.30)	37.1
6	5c	CH ₂ CH ₂ CH ₃	PLE	17	40	−4.1° (c=1.20)	24.3
7	5d	CH ₂ Cl	PLE	2	71	—	—

a) Yield based on converted substrate.

with isopropenyl methyl ether in the presence of pyridinium *p*-toluenesulfonate (PPTS), followed by deacetylation with K₂CO₃–MeOH, **8** was converted in 70% yield into the known

Chart 3

compound (**10**), synthesized and transformed into PAF by Ohno *et al.*¹⁰⁾ Based on the reported value ([α]_D²¹ − 11.3° (c=1.72, MeOH)), the optical purity of our sample ([α]_D²² − 4.3° (c=6.25, MeOH)) was estimated to be 38% ee.

Experimental

Infrared (IR) spectra were measured with a JASCO A-202 spectrometer. ¹H-NMR spectra were measured on a JEOL LNP-PS-100 spectrometer unless otherwise stated. Mass spectra (MS) were taken on a JEOL JMS-D 300 spectrometer. Specific rotations were measured on a JASCO DIP-4 polarimeter. For column chromatography, silica gel (Merck, Kieselgel 60, 70—230 mesh) was used. Thin-layer chromatography (TLC) and preparative TLC were performed on silica gel plates (Merck, Kieselgel 60 F₂₅₄, 0.25 and 2 mm, respectively). For enzymic hydrolysis, PLE

(Sigma, type I), PPL (Sigma, type II (pfs)) and lipase from *Candida cylindracea* (Sigma, type VII (pfs)) were used. All organic solvent extracts were washed with satd. brine and dried on anhydrous sodium sulfate.

1,3-Diacetoxyacetone (3)—Ac₂O (62 g) was added dropwise to a stirred solution of **2** (25.0 g) in pyridine (50 ml) at 5–10 °C. The reaction mixture was stirred for 14 h at room temperature. Pyridine was removed *in vacuo* to afford a crystalline residue, which was washed with hexane and recrystallized from hexane–AcOEt to yield **3** as colorless needles (47.4 g, 98%), mp 45.5–46.6 °C. IR (Nujol): 1770, 1745, 1230 cm⁻¹. ¹H-NMR (CDCl₃) δ: 2.18 (6H, s, OCOCH₃ × 2), 4.76 (4H, s, CH₂ × 2).

1,3-Di-O-acetyl-2-O-benzylglycerol (4)—Ethereal Zn (BH₄)₂ (0.212 M, 320 ml) was added dropwise to a stirred solution of **3** (47.0 g) in tetrahydrofuran (THF) (100 ml) at 10 °C. The reaction mixture was stirred for 15 min at room temperature. Then 10% HCl (22 ml) was added dropwise at 0 °C, and the resultant precipitate was filtered off. The filtrate was dried and concentrated *in vacuo* to afford an oily residue, which was subjected to column chromatography on silica gel (300 g). The fraction eluted with 40% AcOEt in hexane (v/v) afforded **4** (38.5 g, 81%) as a colorless oil, and the fraction eluted with 60% AcOEt in hexane (v/v) yielded (±)-1-O-acetyl-2-O-benzylglycerol (**8**, 4.6 g, 13%) as a colorless oil. **4**: IR (neat): 3470, 1740, 1245, 1050 cm⁻¹. ¹H-NMR (CDCl₃) δ: 2.11 (6H, s, OCOCH₃ × 2), 2.88 (1H, br, OH), 3.72 (1H, m, CH), 4.13 (4H, s, CH₂ × 2). MS *m/z*: 176 (M⁺), 158, 103.

1,3-Di-O-acetyl-2-O-benzylglycerol (5a) and (±)-1-O-Acetyl-2-O-benzylglycerol (6a)—A solution of benzyl chloride (33.6 g) in DMSO (20 ml) was added dropwise to a stirred solution of **3** (36.0 g) and NaH (60% content, 8.18 g) in DMSO (140 ml) at 10 °C. The reaction mixture was stirred for 4.5 h at room temperature, poured into 3% HCl (150 ml) and extracted with AcOEt (250 ml × 2). The combined extracts were washed and dried. Removal of the solvent *in vacuo* gave an oily residue, which was chromatographed on silica gel (600 g). The fraction eluted with 10% AcOEt in hexane (v/v) was collected, and removal of the solvent *in vacuo* afforded **5a** (18.5 g, 34%). The fraction eluted with 20% AcOEt in hexane (v/v) yielded (±)-**6a** (8.8 g, 19%) as a colorless oil. **5a**: IR (neat): 1740, 1500, 1240, 1050 cm⁻¹. ¹H-NMR (CDCl₃) δ: 2.06 (6H, s, OCOCH₃ × 2), 3.78 (1H, tt, *J* = 5, 5 Hz, CH), 4.17, 4.20 (2H each, dd, *J* = 14, 5 Hz, CH₂O × 2), 4.64 (2H, s, CH₂Ph), 7.31 (5H, s, aromatic-H). MS *m/z*: 266 (M⁺), 223, 193. *Anal.* Calcd for C₁₄H₁₈O₅: C, 63.14; H, 6.81. Found: C, 63.25; H, 6.83. (±)-**6a**: IR (neat): 3450, 1740, 1500, 1245, 1050 cm⁻¹. ¹H-NMR (CDCl₃) δ: 2.06 (3H, s, OCOCH₃), 2.20 (1H, br, OH), 3.66 (3H, m, CH, CH₂OH), 4.21 (2H, m, CH₂OAc), 4.64, 4.65 (1H each, d, *J* = 15 Hz, CH₂Ph), 7.32 (5H, s, aromatic-H). MS *m/z*: 224 (M⁺), 181, 164. *Anal.* Calcd for C₁₂H₁₆O₄: C, 64.27; H, 7.19. Found: C, 64.11; H, 7.28.

2-O-Benzylglycerol (7)—K₂CO₃ (415 mg) was added to a stirred solution of **5a** (4.0 g) in MeOH (12 ml) at room temperature. The whole was stirred for 15 min, and AcOH (180 mg) was added. The solvent was removed *in vacuo* to afford an oily residue, which was subjected to column chromatography on silica gel (45 g). The fraction eluted with 50% AcOEt in hexane (v/v) was collected. Removal of the solvent afforded **7** (2.24 g, 82%) as a colorless oil. IR (neat): 3450, 1500, 740, 700 cm⁻¹. ¹H-NMR (CDCl₃) δ: 2.67 (2H, br, OH × 2), 3.63 (5H, m, CH, CH₂ × 2), 4.57 (2H, s, CH₂Ph), 7.25 (5H, s, aromatic-H).

General Procedure for the Preparation of 1,3-Di-O-acyl-2-O-benzylglycerols (5b–d)—Acid anhydride (6.6 mmol) was added dropwise to a stirred solution of **7** (800 mg) in pyridine (3 ml) at 10 °C. The reaction mixture was stirred for 1 h at room temperature, poured into 2% HCl (50 ml) and extracted with AcOEt (100 ml × 2). The combined extracts were successively washed with 5% aq. Na₂CO₃ (50 ml) and satd. brine, then dried. After removal of the solvent *in vacuo*, the crude product was purified by column chromatography on silica gel (20 g).

1,3-Di-O-propionyl-2-O-benzylglycerol (5b)—**7** (800 mg) and propionic anhydride (1.72 g) were used for the reaction, and the crude product was subjected to column chromatography on silica gel. The fraction eluted with 10% AcOEt in hexane (v/v) was collected, and removal of the solvent *in vacuo* afforded **5b** (1.24 g, 96%) as a colorless oil. ¹H-NMR (CDCl₃) δ: 1.13 (6H, t, *J* = 7 Hz, CH₃ × 2), 2.34 (4H, q, *J* = 7 Hz, CH₂CH₃ × 2), 3.81 (1H, tt, *J* = 5, 5 Hz, CH), 4.19, 4.20 (2H each, dd, *J* = 14, 5 Hz, CH₂O × 2), 4.65 (2H, s, CH₂Ph), 7.31 (5H, s, aromatic-H). MS *m/z*: 294 (M⁺), 237, 187.

1,3-Di-O-butyryl-2-O-benzylglycerol (5c)—**7** (800 mg) and butyric anhydride (2.09 g) were used for the reaction, and the crude product was subjected to column chromatography on silica gel. The fraction eluted with 5% AcOEt in hexane (v/v) afforded **5c** (1.16 g, 82%) as a colorless oil. ¹H-NMR (CDCl₃) δ: 0.93 (6H, t, *J* = 7 Hz, CH₃ × 2), 1.64 (4H, tq, *J* = 7, 7 Hz, CH₂CH₂CH₃ × 2), 2.30 (4H, t, *J* = 7 Hz, CH₂COO × 2), 3.79 (1H, tt, *J* = 5, 5 Hz, CH), 4.18, 4.19 (2H each, dd, *J* = 15, 5 Hz, CH₂O × 2), 4.64 (2H, s, CH₂Ph), 7.31 (5H, s, aromatic-H). MS *m/z*: 322 (M⁺), 251, 215.

1,3-Di-O-chloroacetyl-2-O-benzylglycerol (5d)—**7** (1.00 g) and chloroacetic anhydride (2.80 g) were used for the reaction, and the crude product was subjected to column chromatography on silica gel. The fraction eluted with 10% AcOEt in hexane (v/v) gave **5d** (1.56 g, 85%) as a colorless oil. IR (neat): 1765, 1500, 1290, 1170 cm⁻¹. ¹H-NMR (CDCl₃) δ: 3.85 (1H, tt, *J* = 5, 5 Hz, CH), 4.02 (4H, s, CH₂Cl × 2), 4.29, 4.30 (2H each, dd, *J* = 15, 5 Hz, CH₂O × 2), 4.64 (2H, s, CH₂Ph), 7.32 (5H, s, aromatic-H).

General Procedure for PLE-Catalyzed Hydrolysis of Diacylates (5a–d)—PLE (800 units) was added to a stirred suspension of a substrate (**5**, 1.45 mmol) in acetone (4 ml) and phosphate buffer (pH 7.0, 0.05 M) (54 ml). The reaction mixture was stirred for 1.5–7 h at 30 °C. The reaction mixture was extracted with AcOEt (100 ml × 2), and the combined extracts were dried. After removal of the solvent *in vacuo*, the crude product was purified by column

chromatography on silica gel (8 g). The results of the reactions are summarized in Table I. **6b**: $^1\text{H-NMR}$ (CDCl_3) δ : 1.13 (3H, t, $J=7$ Hz, CH_3), 2.20 (1H, br, OH), 2.35 (2H, q, $J=7$ Hz, CH_2COO), 3.66 (2H, s, CH_2OH), 3.76 (1H, m, CH), 4.22 (2H, d, $J=15$ Hz, CH_2OCO), 4.63, 4.65 (1H each, d, $J=15$ Hz, CH_2Ph), 7.32 (5H, s, aromatic-H). MS m/z : 238 (M^+), 207, 181. **6c**: $^1\text{H-NMR}$ (CDCl_3) δ : 0.94 (3H, t, $J=7$ Hz, CH_3), 1.66 (2H, tq, $J=7, 7$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.32 (2H, t, $J=7$ Hz, CH_2COO), 3.67 (2H, s, CH_2OH), 3.74 (1H, m, CH), 4.23 (2H, d, $J=15$ Hz, CH_2OCO), 4.65, 4.66 (1H each, d, $J=15$ Hz, CH_2Ph), 7.32 (5H, s, aromatic-H). MS m/z : 252 (M^+), 221, 181. **6d**: $^1\text{H-NMR}$ (CDCl_3) δ : 3.65 (2H, s, CH_2OH), 3.81 (1H, m, CH), 4.03 (2H, s, CH_2Cl), 4.31 (2H, m, CH_2OCO), 4.64, 4.65 (1H each, d, $J=15$ Hz, CH_2Ph), 7.32 (5H, s, aromatic-H).

PPL-Catalyzed Hydrolysis of 5a—**5a** (532 mg) in phosphate buffer (pH 7.0, 0.1 M) (50 ml) was stirred with PPL (10 mg, 130 units) for 6 h at 30°C , and worked up in the usual manner. The result is included in Table I.

Candida cylindracea Lipase-Catalyzed Hydrolysis of 5a—**5a** (532 mg) in phosphate buffer (pH 7.0, 0.1 M) (50 ml) was stirred with lipase from *Candida cylindracea* (10 mg, 5000 units) for 43 h at 30°C , and worked up in the usual manner. The result is included in Table I.

Baker's Yeast-Catalyzed Hydrolysis of 5a—**5a** (500 mg) was added in a stirred mixture of sucrose (20 g), baker's yeast (20 g) and H_2O (100 ml), and the whole was stirred for 2 h at 30°C . AcOEt (100 ml) was added to the reaction mixture, and the precipitate was filtered off. The aqueous layer of the filtrate was again extracted with AcOEt (100 ml $\times$ 2), and the combined extracts were washed and dried. After removal of the solvent, the crude product was purified by column chromatography on silica gel (8 g). The result is included in Table I.

MTPA Ester of 6a—(–)-MTPA chloride (37 mg) was added to a stirred solution of (–)-**6a** (20 mg) in pyridine (1 ml) at 10°C . The reaction mixture was stirred for 3 h at room temperature, diluted with Et_2O (50 ml), washed with satd. brine and dried. Removal of the solvent *in vacuo* afforded the ester, which was purified by preparative TLC in AcOEt–hexane (1:4) to afford the pure ester (40 mg, 92%) as a colorless oil. The MTPA esters of **6b** and **6c** were similarly synthesized.

1-O-Acetyl-sn-glycerol (8)—(–)-**6a** (302 mg) was subjected to hydrogenolysis under an H_2 atmosphere using 5% Pd–C (200 mg) as a catalyst in EtOH (25 ml). After removal of the catalyst by filtration, the solvent was removed *in vacuo* to afford an oily residue, which was chromatographed on silica gel (2 g). The fraction eluted with 50% AcOEt in hexane (v/v) was collected. Removal of the solvent *in vacuo* afforded **8** (131 mg, 77%) as a colorless oil. $[\alpha]_{\text{D}}^{27} + 0.83^\circ$ ($c=2.78$, MeOH). IR (neat): 3400, 1720, 1260, 1045 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 2.10 (3H, s, OCOCH_3), 2.60, 3.24 (1H each, br, OH $\times$ 2), 3.61 (2H, m, CH_2OH), 3.92 (1H, m, CH), 4.14 (2H, d, $J=6$ Hz, CH_2OAc). MS m/z : 135 ($\text{M}^+ + 1$), 103.

1-O-Acetyl-2, 3-O-isopropylidene-sn-glycerol (9)—PPTS (10 mg) was added to a stirred mixture of **8** (320 mg), 2-methoxypropene (516 mg) and CH_2Cl_2 (1.5 ml) under ice–water cooling. The reaction mixture was stirred for 1 h at room temperature, diluted with CH_2Cl_2 (100 ml), washed with satd. brine (25 ml $\times$ 3), then dried. Removal of the solvent afforded a crude product **8** (364 mg, 88%) as a colorless oil. IR (neat): 1743, 1370, 1230, 1050 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 1.36, 1.42 (3H each, s, $\text{CH}_3 \times 2$), 2.08 (3H, s, CH_3COO), 3.70 (1H, m, CH), 3.93–4.42 (4H, m, $\text{CH}_2\text{O} \times 2$).

2,3-O-Isopropylidene-sn-glycerol (10)— K_2CO_3 (116 mg) was added to a stirred solution of **9** (364 mg) in MeOH (2 ml) at room temperature. The mixture was stirred for 5 h, diluted with AcOEt (150 ml), washed with satd. brine (50 ml $\times$ 3), and dried. Removal of the solvent *in vacuo* afforded an oily residue, which was distilled under reduced pressure to yield **10** (208 mg, 75%) as a colorless oil. bp $110\text{--}125^\circ\text{C}$ (bath temp.)/2 mmHg. $[\alpha]_{\text{D}}^{22} - 4.30^\circ$ ($c=6.25$, MeOH). IR (neat): 3420, 2990, 2930, 1380, 1370 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 1.37, 1.44 (3H each, s, $\text{CH}_3 \times 2$), 2.29 (1H, br, OH), 3.48–4.40 (5H, m, CH, $\text{CH}_2 \times 2$). Anal. Calcd for $\text{C}_6\text{H}_{12}\text{O}_3$: C, 54.53; H, 9.15. Found: C, 54.69; H, 9.26.

References and Notes

- 1) C. A. Demopoulos, R. N. Pinkard, and D. J. Hanahan, *J. Biol. Chem.*, **254**, 9355 (1979).
- 2) B. B. Vargaftig, M. Chignard, J. Benveniste, J. Lefort, and F. Wal, *Ann. N. Y. Acad. Sci.*, **370**, 119 (1981) and references cited therein.
- 3) R. N. Pinkard, L. M. McManus, and D. J. Hanahan, *Adv. Inflammation Res.*, **4**, 147 (1982).
- 4) J. Benveniste, E. Jouvin, E. Pirotzky, B. Arnoux, J. M. Mencia-Huerta, R. Roubin, and B. B. Vargaftig, *Int. Arch. Allergy Appl. Immunol.*, **66** (suppl. 1), 121 (1981).
- 5) H. Suemune, A. Akashi, and K. Sakai, *Chem. Pharm. Bull.*, **33**, 1055 (1985) and references cited therein.
- 6) R. Noyori, I. Tomino, and M. Nishizawa, *J. Am. Chem. Soc.*, **101**, 5843 (1979).
- 7) Kinetic resolution of ($\pm$)-1-O-butyl-2,3-O-isopropylidene-glycerol has been done by an enzymic procedure. W. E. Lander and G. M. Whitesides, *J. Am. Chem. Soc.*, **106**, 7250 (1984).
- 8) a) Y.-F. Wang, C.-S. Chen, G. Girdaukas, and C. J. Sih, *J. Am. Chem. Soc.*, **106**, 3695 (1984); b) Y.-F. Wang and C. J. Sih, *Tetrahedron Lett.*, **25**, 4999 (1984); c) K. Laumen and M. Schneider, *ibid.*, **26**, 2073 (1985).
- 9) A. J. E. Porck and B. M. Craig, *Can. J. Chem.*, **33**, 1286 (1955).
- 10) M. Ohno, K. Fujita, H. Nakai, S. Kobayashi, K. Inoue, and S. Nojima, *Chem. Pharm. Bull.*, **33**, 572 (1985).