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Synthesis and Biological Activity of the Metabolites of [3,4-Bis(4-methoxyphenyl)-5-isoxazolyl]acetic Acid

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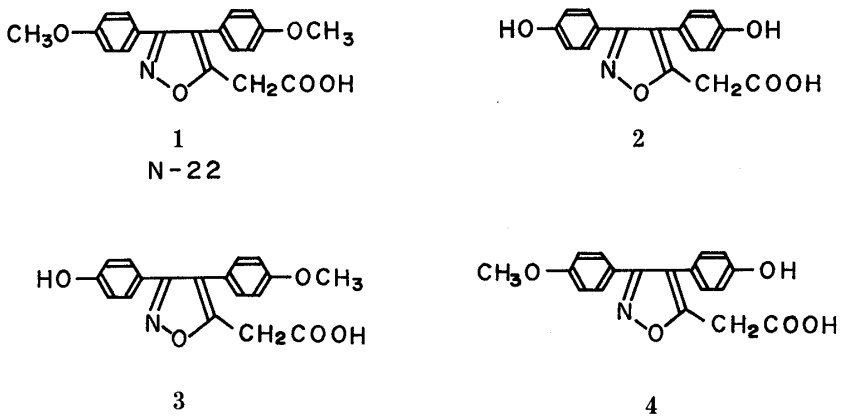
Three metabolites of [3,4-bis(4-methoxyphenyl)-5-isoxazolyl]acetic acid (N-22) (**1**) were prepared and examined for prostaglandin synthetase inhibitory activity. The inhibitory activity of one of these metabolites (**3**) was similar to that of the parent compound (**1**) but the other metabolites (**2** and **4**) showed lower activity.

Keywords——metabolite; [3,4-bis(4-methoxyphenyl)-5-isoxazolyl]acetic acid; prostaglandin synthetase inhibitory activity; [3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-5-isoxazolyl]acetic acid; [4-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-5-isoxazolyl]acetic acid; [3,4-bis(4-hydroxyphenyl)-5-isoxazolyl]acetic acid

[3,4-Bis(4-methoxyphenyl)-5-isoxazolyl]acetic acid (N-22) (**1**), which was synthesized by Micetich *et al.* as a non-steroidal anti-inflammatory agent,[1] is a new derivative of isoxazole having a potent prostaglandin biosynthetase inhibitory activity[2] and is being examined for possible clinical application as an anti-inflammatory agent. In metabolic studies,[3] three major metabolites were isolated from the biological fluid of rats and they were suggested to be compounds demethylated on the phenyl ring. To confirm their structures, we synthesized compounds **2**—**4** as described below, and examined their prostaglandin synthetase inhibitory activity.

Synthesis

[3-(4-Hydroxyphenyl)-4-(4-methoxyphenyl)-5-isoxazolyl]acetic acid (**3**) and [4-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-5-isoxazolyl]acetic acid (**4**) were synthesized by a modification of the method of Micetich *et al.*[1] First, 4-hydroxyphenyl 4-methoxybenzyl ketone or 4-hydroxybenzyl 4-methoxyphenyl ketone (**5a** or **5b**)[4] was treated with dihydropyran in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate ($TsOH \cdot H_2O$)



1 N-22 2 3 4

Fig. 1

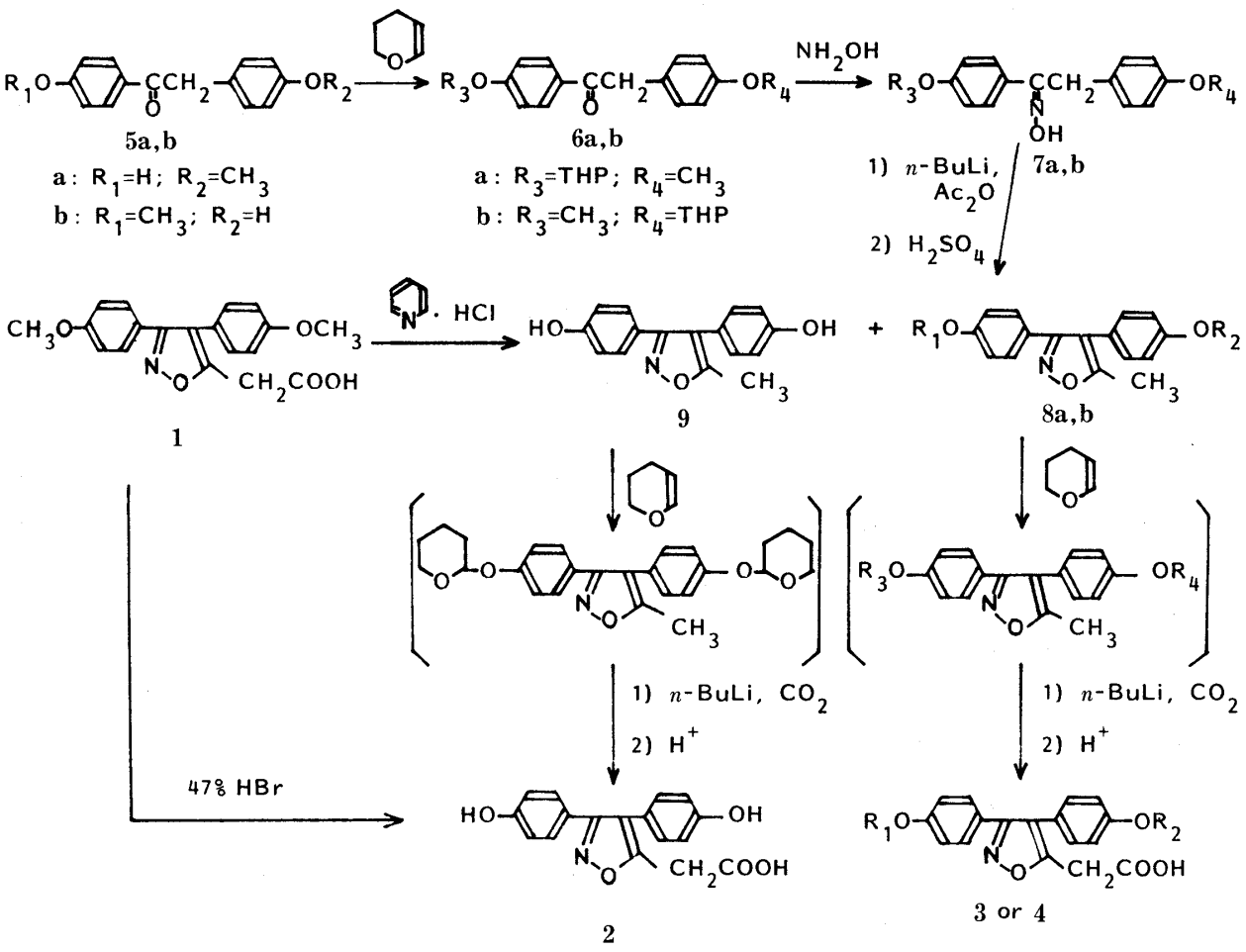


Chart 1

according to the known method[5] to afford the tetrahydropyranyl ether (**6a** or **6b**). Compound **6a** or **6b** was treated with hydroxylamine (NH_2OH) according to the method of Fusco *et al.*[6] to give an oxime (**7a** or **7b**). This compound (**7a** or **7b**) was acetylated using *n*-butyllithium (*n*-BuLi) and acetic anhydride, followed by cyclization with concentrated H_2SO_4, to give an isoxazole derivative (**8a** or **8b**). Finally, **3** or **4** was prepared from **8a** or **8b** protected as the tetrahydropyranyl ether, by treatment with *n*-BuLi and carbon dioxide followed by deprotection of the tetrahydropyranyl ether with HCl. [3,4-Bis(4-hydroxyphenyl)-5-isoxazolyl]-acetic acid (**2**) was prepared from 3,4-bis(4-hydroxyphenyl)-5-methylisoxazole (**9**) by a synthetic procedure similar to that used for **8a** or **8b**. Compound **9** was prepared from [3,4-bis-(4-methoxyphenyl)-5-isoxazolyl]acetic acid (**1**)[1] by treatment with pyridinium chloride according to Filler *et al.*[7] Compound **2** was also obtained by treatment of **1** with 47% HBr according to the method by Long and Burger.[8]

Each metabolite (**2**—**4**) from biological fluids was identical with the corresponding synthetic compound on comparisons of the molecular ion peak and fragmentation in electron impact mass spectrometry (EI-MS) and the retention time on reversed-phase liquid chromatography with a ultraviolet (UV) absorbance detector.

Biological Results

The prostaglandin synthetase inhibitory activity of synthetic metabolites was examined by the method described in the previous paper,[9] and the results are summarized in Table I. The metabolite (**3**) was active but the activity of **2** or **4** was lower than that of **1**. It thus appears that the methoxy moiety of metabolite (**3**) is necessary for inhibitory activity.

TABLE I. Prostaglandin Synthetase Inhibitory Activities of N-22 and Their Metabolites

Compd. No.	Concentration (μM)	n	Inhibition (%) (mean±S.E.)
1	0.1	10	32.2±3.6
	0.3	10	80.0±2.4
2	30	3	39.2±3.6
	100	4	67.6±5.2
3	0.1	4	27.2±2.5
	0.3	4	61.8±2.9
4	1	4	17.2±4.3
	3	4	38.0±5.7

Experimental

All melting points were recorded with a Yanagimoto micromelting point apparatus and are uncorrected. Spectral data were obtained as follows: infrared (IR) spectra with a Hitachi 260-50 spectrophotometer; mass spectra (MS) with a JEOL LMS-01G-2 spectrometer; proton nuclear magnetic resonance (^{1}H-NMR) spectra with a JEOL JMN-FX 100 spectrometer (using tetramethylsilane as the internal standard). Elemental analysis was carried out with a Yanagimoto MT-2 CHN Corder.

4-Methoxybenzyl 4-(2-tetrahydropyranyloxy)phenyl Ketone (6a)——A catalytic amount of $TsOH \cdot H_2O$ was added to a mixture of **5a**[4] (17.0 g, 70.2 mmol) and dihydropyran (90 ml) and the mixture was stirred at room temperature for 6 h. Chloroform was added to the reaction mixture, then the organic layer was washed with saturated $NaHCO_3$ solution and water, dried over $MgSO_4$ and concentrated. Recrystallization from EtOH gave **6a** (18.3 g, 80%) as a colorless powder, mp 128—130 °C. IR (KBr): 1680 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.3—2.0 (6H, m, tetrahydropyran ring), 3.4—3.9 (2H, m, tetrahydropyran ring), 3.71 (3H, s, OCH_3), 4.22 (2H, s, CH_2), 5.61 (1H, br s, tetrahydropyran ring), 6.86 (2H, d, J=8.8 Hz, ArH), 7.11 (2H, d, J=8.8 Hz, ArH), 7.18 (2H, d, J=8.8 Hz, ArH), 8.01 (2H, d, J=8.8 Hz, ArH). *Anal.* Calcd for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79. Found: C, 73.93; H, 6.96.

4-Methoxyphenyl 4-(2-tetrahydropyranyloxy)benzyl Ketone (6b)——Compound **6b** (7.5 g, 80%) was prepared in the same way as described for **6a** using **5b**[4] (7.0 g, 28.9 mmol), dihydropyran (40 ml) and a catalytic amount of $TsOH \cdot H_2O$. Colorless prisms were obtained from MeOH, mp 100—101 °C. IR (KBr): 1680 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.4—2.2 (6H, m, tetrahydropyran ring), 3.4—4.0 (2H, m, tetrahydropyran ring), 3.85 (3H, s, OCH_3), 4.16 (2H, s, CH_2), 5.37 (1H, br s, tetrahydropyran ring), 6.90 (2H, d, J=9.0 Hz, ArH), 7.00 (2H, d, J=9.0 Hz, ArH), 7.20 (2H, d, J=9.0 Hz, ArH), 7.99 (2H, d, J=9.0 Hz, ArH). *Anal.* Calcd for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79. Found: C, 73.70; H, 6.95.

4-Methoxybenzyl 4-(2-tetrahydropyranyloxy)phenyl Ketoxime (7a)——A solution of **6a** (18.0 g, 55.1 mmol) in tetrahydrofuran (THF) (225 ml) was added to a solution of $NH_2OH \cdot HCl$ (5.7 g, 82.0 mmol) and KOH (4.6 g, 82.0 mmol) in EtOH (130 ml) followed by standing overnight at room temperature. After evaporation, the residue was extracted with ether and the organic layer was washed with water, dried over $MgSO_4$ and concentrated. Recrystallization from MeOH gave **7a** (12.0 g, 64%) as a colorless powder, mp 134—136 °C. IR (KBr): 1510 (C=N) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.3—2.0 (6H, m, tetrahydropyran ring), 3.4—3.9 (2H, m, tetrahydropyran ring), 3.67 (3H, s, OCH_3), 4.03 (2H, s, CH_2), 5.45 (1H, br s, tetrahydropyran ring), 6.80 (2H, d, J=8.8 Hz, ArH), 6.97 (2H, d, J=8.8 Hz, ArH), 7.14 (2H, d, J=8.8 Hz, ArH), 7.59 (2H, d, J=8.8 Hz, ArH), 11.23 (1H, s, NOH). *Anal.* Calcd for $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; N, 4.10. Found: C, 70.66; H, 6.83; N, 4.00.

4-Methoxyphenyl 4-(2-tetrahydropyranyloxy)benzyl Ketoxime (7b)——Compound **7b** (5.5 g, 70%) was prepared in the same way as described for **7a** using **6b** (7.5 g, 23.0 mmol), $NH_2OH \cdot HCl$ (1.9 g, 27.3 mmol) and KOH (1.6 g, 28.5 mmol) in EtOH (80 ml). A colorless powder was obtained from MeOH, mp 126—129 °C. IR (KBr): 1510 (C=N) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.3—2.2 (6H, m, tetrahydropyran ring), 3.3—4.2 (2H, m, tetrahydropyran ring), 3.76 (3H, s, OCH_3), 4.12 (2H, s, CH_2), 5.35 (1H, br s, tetrahydropyran ring), 6.84 (2H, d, J=9.1 Hz, ArH), 6.94 (2H, d, J=9.1 Hz, ArH), 7.18 (2H, d, J=9.1 Hz, ArH), 7.58 (2H, d, J=9.1 Hz, ArH), 8.65 and 9.25 (each 1H, br s, br s, NOH). *Anal.* Calcd for $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; N, 4.10. Found: C, 70.48; H, 7.04; N, 4.06.

3-(4-Hydroxyphenyl)-4-(4-methoxyphenyl)-5-methylisoxazole (8a)——A 15% solution of n-BuLi in hexane (36.0 ml, 57.3 mmol) was added dropwise to a solution of **7a** (7.8 g, 22.8 mmol) in dry THF (110 ml) under an argon atmosphere below −10 °C. The reaction mixture was stirred at −10 °C for 1 h, then gradually allowed to reach room temperature, and stirred at room temperature for 1 h. Acetic anhydride (2.5 ml, 26.4 mmol) was added, and the mixture was stirred at room temperature for 1 h. Concentrated H_2SO_4 (14 ml) was then added followed by heating under reflux for 1 h. Chloroform was added to the reaction mixture, and the organic layer was washed with water, dried over $MgSO_4$ and concentrated. Recrystallization from MeOH gave **8a** (2.8 g, 44%) as colorless needles, mp

220 °C. IR (KBr): 3150 (OH) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.37 (3H, s, CH_3), 3.78 (3H, s, OCH_3), 6.75 (2H, d, J=8.8 Hz, ArH), 6.96 (2H, d, J=9.0 Hz, ArH), 7.14 (2H, d, J=9.0 Hz, ArH), 7.19 (2H, d, J=8.8 Hz, ArH), 9.79 (1H, s, OH). MS m/z: 281 (M^+). *Anal.* Calcd for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.83; H, 5.57; N, 4.96.

4-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-5-methylisoxazole (8b)——Compound **8b** (0.2 g, 34%) was prepared in the same way as described for **8a** using **7b** (0.7 g, 2.1 mmol) in dry THF (10 ml), a 15% solution of *n*-BuLi in hexane (4.2 ml, 6.0 mmol), acetic anhydride (0.2 g, 2.0 mmol) and concentrated H_2SO_4 (0.5 ml). Colorless needles were obtained from EtOH, mp 180—182 °C. IR (KBr): 3200 (OH) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.41 (3H, s, CH_3), 3.79 (3H, s, OCH_3), 6.84 (2H, d, J=8.8 Hz, ArH), 6.86 (2H, d, J=9.0 Hz, ArH), 7.05 (2H, d, J=8.8 Hz, ArH), 7.40 (2H, d, J=9.0 Hz, ArH), 9.90 (1H, br s, OH). *Anal.* Calcd for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.72; H, 5.61; N, 4.78.

[3-(4-Hydroxyphenyl)-4-(4-methoxyphenyl)-5-isoxazolyl]acetic Acid (3)——A mixture of **8a** (4.0 g, 14.2 mmol), dihydropyran (24 ml) and a catalytic amount of TsOH·H_2O was allowed to stand overnight at room temperature, and $CHCl_3$ was added to the reaction mixture. The organic layer was washed with saturated $NaHCO_3$ solution and water, dried over $MgSO_4$ and concentrated. The residue was chromatographed on a silica gel column (400 g; eluent, $CHCl_3$). The eluate was concentrated to give the tetrahydropyranyl ether of **8a**. The tetrahydropyranyl ether of **8a** thus obtained was found to contain a small amount of by-product, but was used for the following step without further purification. A 15% solution of *n*-BuLi in hexane (10.0 ml, 15.9 mmol) was added dropwise to a solution of the tetrahydropyranyl ether (5.9 g) in dry THF (50 ml) under an argon atmosphere at −70 °C. The reaction mixture was then stirred at −70 °C for 1 h and poured onto crushed dry ice. Chloroform and dilute HCl were added to the mixture and the organic layer was separated, washed with water and concentrated. The residue was chromatographed on a silica gel column (250 g; eluent, $CHCl_3$: MeOH = 5 : 1). The eluate was concentrated, and the residue was dissolved in a solution of THF (20 ml) and concentrated HCl (0.3 ml), followed by standing overnight at room temperature. The reaction mixture was concentrated and the residue was dissolved in dilute KOH solution. The aqueous layer was washed with ether, acidified with dilute HCl and extracted with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$ and concentrated. Recrystallization from CH_3CN–benzene (1 : 4) gave **3** (1.8 g, 39%) as colorless needles, mp 220—220.5 °C (dec., with foaming). IR (KBr): 1710 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.78 (5H, s, OCH_3, CH_2), 6.75 (2H, d, J=8.8 Hz, ArH), 6.97 (2H, d, J=8.8 Hz, ArH), 7.14 (2H, d, J=8.8 Hz, ArH), 7.20 (2H, d, J=8.8 Hz, ArH), 9.68 (1H, br s, OH), 12.7 (1H, br s, COOH). MS m/z: 325 (M^+). *Anal.* Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.86; H, 4.64; N, 4.30.

[4-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-5-isoxazolyl]acetic Acid (4)——Compound **4** (0.9 g, 35%) was prepared by the same procedure as described for **3** using **8b** (2.2 g, 7.8 mmol), dihydropyran (14 ml), a catalytic amount of TsOH·H_2O and a 15% solution of *n*-BuLi in hexane (4.0 ml, 6.4 mmol). Colorless prisms were obtained from CH_3CN–benzene (1 : 4), mp 102—106 °C (dec., with foaming). IR (KBr): 1720 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.76 (5H, s, OCH_3, CH_2), 6.80 (2H, d, J=8.8 Hz, ArH), 6.95 (2H, d, J=9.0 Hz, ArH), 7.02 (2H, d, J=8.8 Hz, ArH), 7.32 (2H, d, J=9.0 Hz, ArH), 9.68 (1H, br s, OH), 12.9 (1H, br s, COOH). MS m/z: 325 (M^+). *Anal.* Calcd for $C_{18}H_{15}NO_5 \cdot 1/4C_6H_6$: C, 67.92; H, 4.82; N, 4.06. Found: C, 67.48; H, 5.30; N, 3.92.

3-(4-Hydroxyphenyl)-4-(4-methoxyphenyl)-5-methylisoxazole (8a), 4-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-5-methylisoxazole (8b), and 3,4-Bis(hydroxyphenyl)-5-methylisoxazole (9)——A mixture of **1** (25.0 g, 73.7 mmol) and pyridinium hydrochloride (50.0 g, 433 mmol) was heated at 170—180 °C for 4 h. After addition of $CHCl_3$ to the reaction mixture, the insoluble material was collected on a filter, washed with water, and recrystallized from CH_3CN to give **9** (12.8 g, 65%) as a colorless powder, mp 252—255 °C. IR (KBr): 3300 (OH) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.35 (3H, s, CH_3), 6.74 (2H, d, J=8.8 Hz, ArH), 6.78 (2H, d, J=8.8 Hz, ArH), 7.01 (2H, d, J=8.8 Hz, ArH), 7.19 (2H, d, J=8.8 Hz, ArH), 9.60 (1H, br s, OH), 9.76 (1H, br s, OH). *Anal.* Calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 72.03; H, 4.91; N, 5.46. The filtrate was washed with water and concentrated. The residue was chromatographed on a silica gel column (250 g; eluent, MeOH : $CHCl_3$ = 1 : 20) to give firstly **8a** (1.0 g, 5%) and then **8b** (0.7 g, 3%).

[3,4-Bis(4-hydroxyphenyl)-5-isoxazolyl]acetic Acid (2)——a) Compound **2** (0.5 g, 20%) was prepared by the same procedure as described for **3** using **9** (2.1 g, 7.9 mmol), dihydropyran (12 ml), a catalytic amount of TsOH·H_2O, and a 15% solution of *n*-BuLi in hexane (3.3 ml, 5.3 mmol), except that ethyl methyl ketone was used instead of ethyl acetate to extract compound **2** from the aqueous layer. A colorless powder was obtained from CH_3CN, mp 183—186 °C (dec., with foaming). IR (KBr): 1720 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.75 (2H, s, CH_2), 6.75 (2H, d, J=8.8 Hz, ArH), 6.79 (2H, d, J=8.8 Hz, ArH), 7.01 (2H, d, J=8.8 Hz, ArH), 7.21 (2H, d, J=8.8 Hz, ArH), 9.64 (1H, br s, OH), 9.79 (1H, br s, OH), 12.89 (1H, br s, COOH). MS m/z: 311 (M^+). *Anal.* Calcd for $C_{17}H_{13}NO_5 \cdot 1/2CH_3CN$: C, 65.16; H, 4.40; N, 6.33. Found: C, 65.45; H, 4.38; N, 6.44.

b) A mixture of **1** (2.5 g, 7.4 mmol) and 47% HBr (10 ml) in AcOH (10 ml) was heated under reflux for 5 h. After evaporation, the residue was extracted with methyl ethyl ketone and the organic layer was washed with water, dried over $MgSO_4$ and concentrated. Recrystallization from CH_3CN gave **2** (0.8 g, 35%), mp 183—185 °C (dec., with foaming).

Biological Method——The inhibition of prostaglandin E_2(PGE_2) formation by sheep seminal vesicle microsomes (SSVM) was measured according to the method of Ono *et al.*[9]

References

1) R. G. Micetich, C. C. Shaw and R. B. Rastogi, Eur. Patent 26928 (1981) [*Chem. Abstr.*, **95**, 115518*d* (1981)].
2) N. Ono, N. Yamamoto, A. Sunami, Y. Yamasaki, H. Ajioka and H. Miyake, Abstract of Papers, 10th International Congress of Pharmacology, Sydney, August 1987, p. 520.
3) T. Marunaka and M. Maniwa, *J. Chromatogr.*, **422**, 227 (1987).
4) D. Lednicer and M. F. Grostic, *J. Org. Chem.*, **32**, 3251 (1967).
5) *a*) W. E. Parham and E. L. Anderson, *J. Am. Chem. Soc.*, **70**, 4187 (1948); *b*) P. D. Klimstra and R. E. Counsell, *J. Med. Chem.*, **8**, 48 (1965).
6) R. Fusco and F. Mazzuchi, *Gazz. Chim. Ital.*, **71**, 406 (1941).
7) R. Filler, B. T. Khan and C. W. McMullen, *J. Org. Chem.*, **27**, 4660 (1962).
8) L. Long, Jr. and A. Burger, *J. Org. Chem.*, **6**, 852 (1941).
9) N. Ono, Y. Yamasaki, N. Yamamoto, A. Sunami and H. Miyake, *Jpn. J. Pharmacol.*, **42**, 431 (1986).