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Synthesis of Mefenamic Acid Derivatives and Antioxidative and Anticoagulant Activities

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Abstract – Mefenamic acid has been widely used as clinical drug for anti-inflammatory and analgesic. This drug was known to non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen and indomethacin. Although the drugs which comprise this group are of diverse chemical structures, they all share the antipyretic, analgesic and anti-inflammatory actions which are characteristic of aspirin. Action of this drugs is caused by inhibitory effect of biosynthesis of prostaglandin that are synthesized from arachidonic acid via the endoperoxide biosynthesis pathway, the initial step of which is catalysed by cyclooxygenase. Mefenamic acid has more potent inhibitory action of prostaglandin biosynthesis than aspirin. Therefore, mefenamic acid is expected to have anticoagulant activity as aspirin-like drugs. This study was carried out to investigate the synthesis of mefenamic acid derivatives from mefenamic acid and aromatic compound of antioxidant and its antioxidative and anticoagulant activities. Synthesis of mefenamic acid derivatives was conformed by conjugation as using esterification method. Biological activities was examined using effect of anticoagulant on bleeding time and effect of antioxidant by TBA method. As a result, SJ-202 showed strong antioxidative activity and anticoagulant activity among tested 4 compounds and exhibited similar activity to aspirin at anticoagulant activity.

Key words ☐ Mefenamic acid, Mefenamic acid derivatives, Aromatic compounds, Antioxidative activity, Anticoagulant activity

Mefenamic acid has been widely used as anti-inflammatory and analgesic drug. However, mefenamic acid was recently utilized not only to reduce inflammatory and fever but also to cure dysmenorrhoea (Delgado *et al.*, 1994), menorrhagia (Van-Eijkeren, *et al.*, 1992) and migraine (Pfaffenrath and Scherzer, 1995). The others, research of therapeutic effect of mefenamic acid against cancer by inhibiting the biosynthesis of prostaglandin was progressed (Duffy, *et al.*, 1998; August, *et al.*, 1994; Teicher, *et al.*, 1994). Mefenamic acid which is drug of anthranilic group like various fenimate, is representative drug of NSAIDs in common with aspirin of salicylic group and ibuprofen of indole group. NSAID was known as a anti-inflammatory drug caused by inhibition of formation of prostaglandin from arachidonic acid through interception of cyclooxygenase action, especially in the first enzymatic step of pathway, during the prostaglandin biosynthesis (Takeguchi and Sih, 1972; Flower, 1974; Vane and Botting, 1987). Among them, mefenamic acid has been reported to reduce the biosynthesis of prostaglandin by competitive inhibitory with cyclooxygenase (Cushman and Cheung, 1976). Besides, Anti-inflammatory activity of indomethacin was resulted from inhibitory of cyclooxygenase-1, while effect of mefenamic acid was caused by inhibitory of cyclooxygenase-2 as isomer of cyclooxygenase-1 (Cryer and Feldman, 1998). Generally, this NSAID has been known to have a side effects as gastrointestinal pathology when used high dose for treatment (Trewin *et al.*, 1994), by the way aspirin and indomethacin at the low dose was found to possess effect of improvement of blood circulation which is caused by anti-aggregation activity through inhibition of excessive formation of thromboxane A2 in platelet followed interception of cyclooxygenase (Bowery and Lewis, 1973; Smith *et al.*, 1976). Since it is so, mefenamic acid may be expected to be a effective improvement of blood circulation when used low dose, as a proof of this hypothesis, it has been shown that mefenamic acid has a action of anti-aggregation by antagonist action of divalent cation channel using 0.1~10 microM (Rho *et al.*, 1995) and had effect of blood prolongation of war-

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farin ascended (Chan, 1995). Therefore, this study was carried out to investigate the synthesis of mefenamic acid derivatives from mefenamic aicd and phenolic compounds of antioxidant by radical scavenging and to estimate its biological activity as a research of development of new drug active against various adult disease.

MATERIAL AND METHOD

NMR spectra were measured by Bruker AC200 spectrometer. Chemical shifts were reported in ppm (δ) relative to TMS as internal standard. Infrared spectra were recorded on a MB-100 FT-IR spectrophotometer in KBr disks. UV spectra were obtained with a Milton Roy Genesis 5 spectrophotometer. Melting points were measured on a Mettler FP 5 and uncorrected. Mefenamic acid, sesamol, eugenol, cinnamyl alcohol, 7-hydroxy-4-methylcoumarin and mesitol were obtained from Aldrich Chemical Company (St. Louis, MO, USA). 1,1'-Carbonyldiimidazole and dimethylaminopyridine were purchased from Sigma Chemical Company (St. Louis, MO, USA). Solvents and other reagents were used of extra pure grade and obtained from local suppliers.

2-[(2,3-Dimethylphenyl)amino]-(4-allyl-2-methoxyphenyl)-benzoate (SJ-201, 1)

A stirred solution of mefenamic acid (6 g, 25 mmol) in DMF (dimethylformamide, 80 ml) was treated addition 1,1'-carbonyldiimidazole (7 g, 0.043 mol) and DMAP (dimetylaminopyridine, 0.219 g, 1.8 mmol) at ice-water bath temperature then allowed to stir for 30 min at room temperature. The mixture was added a eugenol (3 g, 0.018 mol) at room temperature and stirring was continued for an additional 12 h. The reaction mixture was quenched with water and extracted with ethylacetate and the combined organic extracts were washed with 5% HCl, saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give a mixture of product. A given product was recrystallized from Hexane-Ether (1 : 1.5 and 3 : 1) to give SJ-201 {2-[(2,3-dimethylphenyl)amino]-(4-allyl-2-methoxyphenyl)benzoate, 5.72 g} of white solid (82%). : mp 70~72°C; R_f=0.31 (n-Hexane : Acetone =2 : 1); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.21 (3H, s, CH_3), 2.35 (3H, s, CH_3), 3.45 (2H, d, $-CH_2-$), 3.85 (3H, s, OCH_3), 5.21 (2H, t-like, $=CH_2$), 6.02 (1H, m, -CH-), 6.74-8.30 (10H, m, $C_6H_3+C_6H_4+C_6H_3$), 9.23 (1H, s, -NH-); IR (KBr)cm^{-1} 1695 (CO), 1604, 1420 (C=C)

2-[(2,3-Dimethylphenyl)amino]-(3,4-methylenedioxyphenyl)benzoate (SJ-202, 2)

A stirred solution of mefenamic acid (3 g, 0.013 mol) in DMF (50 ml) was treated addition 1,1'-carbonyldiimidazole (1.5 g, 0.0093 mol) and DMAP (0.183 g, 0.0015 mol) at ice-water bath temperature then allowed to stir for 30 min at room temperature. The mixture was added a sesamol (2 g, 0.015 mol) at room temperature and stirring was continued for an additional 12 hr. The reaction mixture was quenched with water and extracted with ethylacetate and the combined organic extracts were washed with 5% HCl, saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give a mixture of product. A given product was recrystallized from Hexane-Ether (1 : 1) to give SJ-202{2-[2,3-dimethylphenyl)amino]-(3,4-methylenedioxyphenyl)benzoate, 4.55 g} of white solid (84%). : mp 118~120°C; R_f=0.81 (n-Hexane : Acetone = 2 : 1); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.16 (3H, s, CH_3), 2.32 (3H, s, CH_3), 6.01 (2H, d, $-CH_2-$), 6.70~8.15 (10H, m, $C_6H_3+C_6H_4+C_6H_3$), 9.28 (1H, s, -NH-); IR (KBr)cm^{-1} 1705 (CO), 1590, 1449 (C=C)

2-[(2,3-Dimethylphenyl)amino]-(3-phenyl-2-propenyl)-benzoate (SJ-203, 3)

A stirred solution of mefenamic acid (3.5 g, 0.015 mol) in DMF (50 ml) was treated addition 1,1'-carbonyldiimidazole (3.6 g, 0.022 mol) and DMAP (0.183 g, 0.0015 mol) at ice-water bath temperature then allowed to stir for 30 min at room temperature. The mixture was added a cinncmyl alcohol (2 g, 0.015 mol) at room temperature and stirring was continued for an additional 12 hr. The reaction mixture was quenched with water and extracted with ethylacetate and the combined organic extracts were washed with 5% HCl, saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give a mixture of product. A given product was recrystallized from Hexane-Ether (6 : 1) to give SJ-203{2-[(2,3-dimethylphenyl)amino]-(3-phenyl-2-propenyl)benzoate, 4.40 g} of white solid (82%). : mp 76~77°C; Rf = 0.64 (n-Hexane : Acetone = 1 : 1); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.33 (3H, s, CH_3), 2.47 (3H, s, CH_3), 5.11 (2H, d, $-CH_2-$), 6.59 (1H, m, -CH), 6.81 (1H, d, -CH), 6.87~8.18 (12H, m, $C_6H_3+ C_6H_4+C_6H_5$), 9.40 (1H, s, -NH-); IR (KBr)cm^{-1} 1680 (CO), 1607, 1450 (C=C)

2-[(2,3-Dimethylphenyl)amino]-(4-methylcoumarinyl)benzoate (SJ-204, 4)

A stirred solution of mefenamic acid (2.7 g, 0.011 mol) in

DMF (50 ml) was treated addition 1,1'-carbonyldiimidazole (1.5 g, 0.0093 mol) and DMAP (0.134 g, 0.0011 mol) at ice-water bath temperature then allowed to stir for 30 min at room temperature. The mixture was added a 7-hydroxy-4-methylcoumarin (2 g, 0.011 mol) at room temperature and stirring was continued for an additional 12 hr. The reaction mixture was quenched with water and extracted with ethylacetate and the combined organic extracts were washed with 5% HCl, saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give a mixture of product. A given product was recrystallized from Hexane-Ether (3 : 1) to give SJ-204{2-[(2,3-dimethylphenyl)amino]-(4-methylcoumarinyl)benzoate, 3.52 g} of white solid (80%). : mp 91~93°C; Rf=0.43 (n-Hexane : Acetone = 1 : 1); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.20 (3H, s, CH_3), 2.35 (3H, s, CH_3), 2.48 (3H, s, CH_3), 6.32 (1H, m, -CH), 6.77~8.22 (10H, m, C_6H_3+ C_6H_4+C_6H_3), 9.12 (1H, s, -NH-); IR (KBr) cm^{-1} 1695 (CO), 1607, 1420 (C=C)

2-[(2,3-Dimethylphenyl)amino]-(2,4,6-trimethylphenyl)benzoate (SJ-205, 5)

A stirred solution of mefenamic acid (3.5 g, 0.015 mol) in DMF (50 ml) was treated addition 1,1'-carbonyldiimidazole (3.6 g, 0.022 mol) and DMAP (0.183 g, 0.0015 mol) at ice-water bath temperature then allowed to stir for 30 min at room temperature. The mixture was added a mesitol (2,4,6-trimethylphenol, 2 g, 0.015 mol) at room temperature and stirring was continued for an additional 12 hr. The reaction mixture was quenched with water and extracted with ethylacetate and the combined organic extracts were washed with 5% HCl, saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give a mixture of product. A given product was recrystallized from Hexane-Ether (2.5 : 1) to give SJ-205{2-[(2,3-dimethylphenyl)amino]-(2,4,6-trimethylphenyl)benzoate, 4.42 g} of white solid (82%). : mp 146~148°C; Rf=0.58 (n-Hexane : Acetone = 1 : 1); ^{1}H-NMR (200 MHz, $CDCl_3$) δ2.32 (3H, s, CH_3), 2.35 (3H, s, CH_3), 2.36 (3H, s, CH_3), 2.38 (3H, s, CH_3), 2.48 (3H, s, CH_3), 6.89~8.43 (9H, m, C_6H_3+ C_6H_4+C_6H_2), 9.40 (1H, s, -NH-); IR (KBr) cm^{-1} 1680 (CO), 1595, 1450 (C=C)

Antioxidative activity

To assess the *in vitro* antioxidative activity of mefenamic acid derivatives, the procedure of Masugi's method (Masugi and Nakamura, 1977) was employed with some modification. The reaction mixture, prepared from 0.3 ml of rat liver homogenate and 0.1 ml of the sample dissolved in 10% DMF in saline, was incubated at 37.5°C for 3 hr. To this resulting mixture was added a 3.6 ml of TBA (thiobarbituric acid) reagent[0.3% TBA and 0.4% SDS (sodium dodecyl sulfate) in 7.5% acetic acid, pH 4.0] and the mixture was heated to 98°C for 1 hr. After cooling, the TBA pigment was extracted with 4 ml of butyl alcohol and the butyl alcohol layer was measured by UV spectrophotometer at 534 nm.

Table 1. Antioxidant effects of mefenamic acid derivatives and aromatic compounds.

Treatment	O.D	Inhibition (%)
Control	2.240 ± 0.051	
BHA	0.576 ± 0.108	74.29
Mefenamic acid	1.812 ± 0.019	19.13
Eugenol	1.180 ± 0.201	47.32
Sesamol	0.261 ± 0.046	88.35
Cinnamyl alcohol	1.430 ± 0.593	36.18
7-Hydroxy-4-methylcoumarin	1.323 ± 0.254	40.92
Mesitol	0.376 ± 0.048	83.20
SJ-201	0.861 ± 0.005	61.58
SJ-202	0.343 ± 0.005	84.67
SJ-203	1.561 ± 0.189	30.31
SJ-204	0.410 ± 0.108	81.71
SJ-205	0.777 ± 0.245	65.33

Concentration : 1.610^{-4} (mol/). Inhibition (%) of MDA generation in rat liver homogenate *in vitro* on TBA method. Values are meansS.D. from 3 separate experiments.

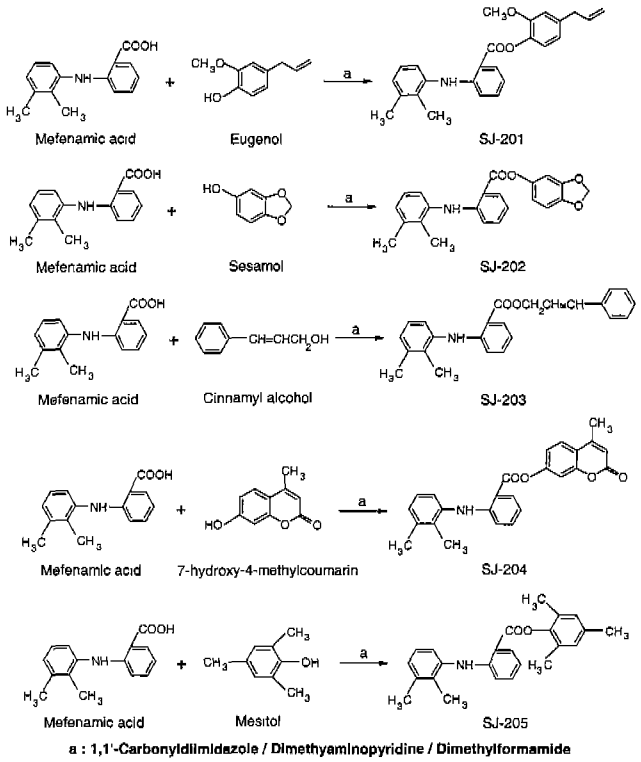
Bleeding time test

The bleeding times of mefenamic acid derivatives and aspirin known effect of excellent bleeding time prolongation (Underwood and More, 1994) as control treated rats were measured as described by Hornstra's method (Hornstra *et al.*, 1981). Drug's of 20 mg/kg were suspended in 1% CMC and given orally once a day for 10 days. At the following day of the last medication, animals were anaesthetized with sodium phenobarbital (50 mg/kg, intraperitoneally). The tail was transsected at 3 mm from the tip, and the tail was immersed 5 cm deep vertically in saline at 37°C. The period between transsection and the moment the third bleeding stopped was taken as the bleeding time. Data are present as mean±S.E. and were analyzed student's t-test by SPSS package. Differences were considered significant when $p<0.05$.

RESULTS AND DISCUSSION

Chemistry

Synthesis of mefenamic acid derivatives was trouble when



Scheme 1. Synthesis of mefenamic acid derivatives from mefenamic acid and aromatic compound by 1,1'-carbonyldiimidazole.

DCC (1,3-dicyclohexylcarbodiimide) was used as a general reagent for esterification because of a weak reaction of phenolic hydroxyl moiety. Therefore, mefenamic acid derivatives were prepared from mefenamic acid and phenolic compounds by esterification using 1,1'-carbonydiimidazole (scheme 1). Chemical structures of SJ-201, 202, 203, 204 and 205 as mefenamic acid derivatives were elucidated by identification of aromatic proton, methyl proton and methylene proton as specification of aromatic compouds proton and none hydroxyl group of each aromatic compounds and amino proton of mefenamic acid by analytical instruments.

Antioxidant activity

Antioxidative activity in *vitro* of mefenamic acid derivatives and each aromatic compounds was shown in Table 1. SJ-202, SJ-204, sesamol and mesitol showed significant inhibitory effect of MDA (malondealdehyde) generation to comparable the value for BHA of synthesis antioxidant. Phenolic compound such as sesamol and mesitol excepting eugenol were shown an antioxidative activity according to expectation. It was thought that antioxidative activity of SJ-202 was induced by sesamol that is formed as hydrolysis of the ester bond by esterase of liver. This result is similar as antioxidative activity of aspirin derivatives that is presentated in the previous our report (Cha and Lee, 2000). But SJ-205 which was synthesized from mesitol was shown weak antioxidative activity. Others, in spite of 7-hydroxy-4-methylcoumarin known as aromatic compound exhibited weak antioxidative activity, its derivative SJ-204 was shown significant antioxidative activity. This result mean that hydrolysis level of the ester bond and antioxidant potency of mefenamic acid derivatives may be due to the structural difference of derivative compounds. Therefore, it is thought that more detailed study of structure-activity relationships of derivative compounds will be needed.

Bleeding time prolongation

NSAID was known as drug that it is possessed effect of bleeding time prolongation caused inhibition of excessive production of thromboxane A2 by interception of cyclooxygenase as enzyme of transfer arachidonic acid to prostaglandin. Aspirin is estimated as the most useful drug among the NSAIDs. Therefore, research of synthesis of aspirin and salicylic acid derivatives and it's biological activity have been progressed (Casadebai *et al.*, 1991; Cha and Lee, 2000). Mefenamic aicd has a inhibitory effect of production of thromboxane A2 as like aspirin (vane, and Botting, 1992). Therefore, research of synthesis of mefenamic acid derivatives and it's biological activity have a mean from the view point of development of a new biological active compound. Whereas the bleeding time of aspirin treated group was increased by 55% (523±51 (s), n=5, $p<0.01$) compared to that of the control group (338±38, n=5), SJ-202 treatment prolonged the bleeding time by 51% (512±66, n=5, $p<0.01$)

Table 2. Effects of anti-coagulant of mefenamic acid derivatives on bleeding time.

Treatment	Bleeding time
Control	338 ± 38
Aspirin	523 ± 51**
Mefenamic acid	475 ± 28**
SJ-201	485 ± 30**
SJ-202	512 ± 48**
SJ-203	380 ± 67
SJ-204	404 ± 26*
SJ-205	502 ± 58**

Values are meanSD (n=5). Bleeding time of adult male rats administrated orally (20mg/kg/day)with various compounds for 10 days. Significantly different with respect to control : *$p<0.05$ **$p<0.01$ (t- test)

and SJ-205 treatment prolonged the bleeding time by 49% (502±58, n=5, $p<0.05$) at the same dose of 20 mg/kg for 10 days (Table 2). It is suggested that the result was derived from synergic effect of bleeding time prolongation caused inhibition of production of tromboxane A2 by inhibition activity of cyclooxygenase action of mefenamic acid and free radical scavenging activity of sesamol and inhibition effect of prostaglandin cyclooxygenase of phenolic compounds (Dewhirst, 1980), but less potent effect of bleeding time prolongation than aspirin.

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