Arch Pharm Res Vol 23, No 2, 116-120, 2000 Medicinal Chemistry & Natural Products

Synthesis and Biological Activity of Aspirin Derivatives

Bae Cheon Cha and Seung Bae Lee

Department of Applied Animal Sciences, College of Life Sciences and Natural Resources, Sangji University, Wonju 220-702, Korea

(Receiveed July 7, 1999)

Aspirin has been widely used as analgesic and anti-inflammatory drug. Recently, it was elucidated that aspirin have anti-coaggregatory effect in low dose. This study was carried out to investigate the synthesis of aspirin derivatives from aspirin and aromatic compound of antioxidant and its biological activities. Synthesis of aspirin derivatives was prepared by esterification in the presence of 1,1-carbonyldiimidazole. Biological activities was examined using effect of anti-coagulant on bleeding time, effect of antioxidant and effect of anti-platelet aggregation. As a result, SJ-101 showed strong antioxidative activity and anti-coagulant activity among four compounds. Anti-platelet aggregation of SJ-101 was examined by collagen, ADP, PAF method. SJ-101 exhibited more stronger activity to aspirin at collagen aggregation reaction. These finding demonstrates that SJ-101 is usefull as care drug of aging and old-disease because of its has antioxidant activity, anti-coagulant activity and anti-platelet activity.

Key words: Aspirin, Aromatic compound, Antioxidant, Anti-coagulant, Anti-platelet

INTRODUCTION

Aspirin has been used as treatment drugs for inflammatory and fever diseases for almost a century. Lately, the biochemical mechanism of action of aspirin was made by Van (Vane, 1971; Flower *et al.*, 1972; Vane and Botting, 1987). Vane proved that aspirin prevented the formation of prostaglandins, he demonstrated the reasons for the anti-inflammatory, analgesic, and toxic effects of the most widely used remedy of all time. More recently, yet another use for aspirin has emerged connected with the discovery of its anti-thrombotic action, which is also base on inhibition of cyclooxygenase enzyme in platelets which makes thromboxane A2, the potent, pro-aggregatory prostaglandin released from platelets (Patrono, 1989). The anti-platelet effects of aspirin have been tested in all forms of coronary arterydisease, pregnancy-induced hypertension and pre-eclampsia in angiotensin-sensitive primigravidae at low dosage (Schoemaker *et al.*, 1998; Wallenburg et al., 1986).

Somewhat, its has been known that diseases related with aging or adult disease result, at least in part, from free radicals as superoxide, hydroxyl radical (Fukuzawa and Takaishi, 1990). Therefore, antioxidant compounds may reduce or prevent the abnormalities associated with free radical formation *via* its scavenging action. In present, enzyme antioxidants as SOD(superoxide dismutase), catalase, glutathione, synthetic antioxidant such as troxol and natural product antioxidant such as tocopherol, ascorbic acid were known widely. The others, the many studies of antioxidant compounds from natural product were reported (Kitahara *et al.*, 1992; Hatano, 1995; Masaki *et al.*, 1995). Out of the many antioxidant compounds, aromatic compound such as phenolic aromatic compounds were known that they have radical scavenging effect. Accordingly, we thought that development of new drugs for aging and adult diseases is possible from conjugation of aspirin and aromatic compounds. This present study was carried out to investigate the synthesis of aspirin derivatives from aspirin and phenolic compounds as antioxidant and their biological activities.

MATERIALS AND METHODS

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. Mass spectrometric data determined by use of the electron impact(EIMS) method with a Finnigan Mat TSQ-700 spectrometer were reported as *m/z*(relative intensity). Melting points were measured

Correspondence to: Bae Cheon Cha, Department of Applied Animal Sciences, College of Life Sciences and Natural Resources, Sangji University, Wonju 220-702, Korea
E-mail: bccha@chiak.sangji.ac.kr

on a Mettler FP 5 and uncorrected. Salicylic acid, sesamol, eugenol, cinnamyl alcohol and 7-hydroxy -4-methylcoumarin were obtained from Aldrich Chemical Company (St. Louis, MO, USA). Reagent of reaction as 1,1-Carbonyldiimidazole and dimethylamino pyridine 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.

3',4'-Methylenedioxyphenyl 2-acetoxybenzoate (SJ-101, 1)

A stirred solution of salicylic acid(2 g, 0.014 mol) in DMF(dimethylformamide, 20 ml) was added with 1,1-carbonyldiimidazole(2.27 g, 0.014 mol) and DMAP (dimethylaminopyridine, 0.171 g, 0.0014 mol) at ice-water bath temperature then allowed to stir for 30 min at room temperature. The mixture was added a sesamol(1.24 g, 0.009 mol) at room temperature and stirring was continued for an additional 12 h. The reaction mixture was quenched with water and extracted with ethyl acetate 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 products. To a solution of a mixture of product in pyridine(10 ml) was added dropwise anhydrous acetic acid(5 ml) at room temperature and stirring was continued for an additional 12 h. The reaction mixture of acetylation was diluted with ethyl acetate, water at ice-water bath temperature and H_2O, the organic layer was separated, washed with saturated $NaHCO_3$, brine, dried over anhydrous $MgSO_4$ and filtered. The filtrate was evaporated *in vacuo* and solid was recrystallized from hexane-ether(1:1) to give 2.754 g of SJ-101 (3',4'-methylenedioxyphenyl 2-acetoxybenzoate) as a white solid (85%). : mp 109 - 111°C; Rf=0.48 (*n*-hexane : acetone = 2 : 1); ^{1}H-NMR(200MHz, $CDCl_3$) δ 2.30(3H, s, OAc), 5.99 (2H, s. $-CH_2-$), 6.53-6.87(3H, m, C_6H_3), 7.09-8.24(4H, m, C_6H_4); IR(KBr)cm^{-1} 1763, 1735, 1604(CO), 1482(C=C); UV λ_{max}nm(log ε) 211(1.08), 231(1.23), 286(0.51); MSm/z(rel.int.) 300(M^+, 11.2), 163(M-CH_3COO -C_6H_4CO, 73.5), 121(M-C_6H_5COO, 100), 43(CH_3CO, 15.2).

4'-Allyl-2'-methoxyphenyl 2-acetoxybenzoate (SJ-102, 2)

To a solution of salicylic acid(2 g, 0.014 mol) in DMF (dimethylformamide, 20 ml) was added a 1,1-carbonyldiimidazole(2.27 g, 0.014 mol) and DMAP (dimethylaminopyridine, 0.171 g, 0.0014 mol) at ice-water bath temperature then allowed to stir for 30 min at room temperature. The mixture was added eugenol (1.2 g, 0.007 mol) at room temperature and stirring was continued for an additional 12 h. The work-up procedure was followed by the above steps in the preparation of SJ-101. Final solid was recrystallized from *n*-hexane-ether(1:1) to give 2.624 g of SJ-102 (4'-allyl-2'-methoxyphenyl 2-acetoxy-benzoate) of white powder(82%): mp 70-71°C; Rf = 0.51 (*n*-hexane : acetone = 2 : 1); ^{1}H-NMR(200MHz, $CDCl_3$) δ 2.30(3H, s, OAc), 3.39(2H, d, $-CH_2-$), 3.81(3H, s, OCH_3), 509(1H, s, $=CH_2$), 5.15(1H, d, $=CH_2$), 5.95(1H, m, CH), 6.79-6.85(3H, m, C_6H_3), 7.09-8.23 (4H, m, C_6H_4); IR(KBr) cm^{-1} 2944(CH), 1764, 1729, 1607(CO), 1424(C=C); UV λ_{max}nm(log ε) 231(0.75), 277(0.18); MSm/z(rel.int.) 326(M^+, 5.2), 164 ($C_{10}H_{12}O_2$, 81.5), 163(M-CH_3COO-C_6H_4 CO, 98), 121(M-C_6H_5COO, 100).

3'-Phenyl-2'-propenyl 2-acetoxybenzoate(SJ-103, 3)

A stirred solution of salicylic acid(2 g, 0.014 mol) in DMF(dimethylformamide, 20 ml) was treated addition with 1,1-carbonyldiimidazole(2.27 g, 0.014 mol) and DMAP (dimethylaminopyridine, 0.171 g, 0.0014 mol) at ice-water bath temperature then allowed to stir for 30 min at room temperature. The mixture was added a cinnamyl alcohol(1.2 g, 0.009 mol) at room temperature and stirring was continued for an additional 12 h. The work-up procedure was followed by the above steps in the preparation of SJ-101. Final solid was recrystallized from *n*-hexane-ether(1:1) to give 2.656 g of SJ-103 (3'-phenyl-2'-propenyl 2-acetoxybenzoate) as a white solid (83%): mp 59 - 60°C; Rf = 0.54 (*n*-hexane : acetone = 2 : 1); ^{1}H-NMR(200MHz, $CDCl_3$) δ 2.31 (3H, s, OAc), 4.92(2H, d. $-CH_2-$), 6.35(1H, m, -CH), 6.71(1H, d, -CH), 7.09 - 8.23(9H, m, $C_6H_4+C_6H_5$); IR(KBr)cm^{-1} 1755, 1719, 1605 (CO), 2948 ; UV λ_{max}nm (log ε) 250(0.26); MSm/z (rel.int.) 296(M^+, 21.1), 253(M-CH_3CO, 41.2), 163(M-$CH_3COOC_6H_4CO$, 100), 121(M-C_6H_5COO, 54.5).

4'-Methylcoumarinyl 2-acetoxybenzoate(SJ-104, 4)

To a solution of salicylic acid(2 g, 0.014 mol) in DMF (dimethylformamide, 20 ml) was added a 1,1-carbonyldiimidazole (2.27 g, 0.014 mol) and DMAP (dimethylaminopyridine, 0.171 g, 0.0014 mol) at ice-water bath temperature then allowed to stir for 30 min at room temperature. The mixture was added 7-hydroxy-4-methyl-coumarin (2.4 g, 0.014 mmol) at room temperature and stirring was continued for an additional 12 h. The work-up procedure was followed by the above steps in the preparation of SJ-101. Final solid was recrystallized from *n*-hexane-ether(1:1) to give 3.564 g of SJ-104 (4'-methyl-coumarinyl 2-acetoxybenzoate) as a white powder (81%) : mp 130-131°C; Rf=0.43 (*n*-hexane : acetone=2 : 1); ^{1}H-NMR (200 MHz, $CDCl_3$) 2.29(3H, s, OAc), 2.38 (3H, s, CH3), 6.20 (1H, s. -CH), 6.98-7.91(7H, m, $C_6H_4+C_6H_5$); IR(KBr)cm^{-1} 2935(CH), 1765, 1727, 1625(CO); UV λ_{max} nm (log ε) 277(0.27); MSm/z(rel.int.) 338(M^+, 9.5), 163(M-$CH_3COOC_6H_4CO$, 67.3.), 121(M-C_6H_5COO, 100).

Antioxidant activity

To assess the *in vitro* antioxidant activity of aspirin derivatives, the procedure of Masugi's method was em-

ployed with some modification (Masugi and Nakamura, 1977). 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 for 3 h. 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 for 1 h. After cooling, the TBA pigment was extracted with 4 ml of buthyl alcohol and the buthyl alcohol layer was measured by UV spectrophotometer at 534 nm.

Bleeding time test

The bleeding times of aspirin 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 $p<0.05$.

Anti-platelet aggregation activity

PRP(platelet rich plasma)was prepared from rabbit blood drawn into syringes containing 0.32% trisodium citrate. The procedure consisted of centrifugation of blood for preparation of PRP, followed by centrifugation of PRP, and resuspension of the platelet pellet in tyrode solution contained 0.35% bovine serum albumin and glucose as described method earlier(Ko *et al.*, 1996). A 315 μl of PRP or diluted solution of platelet pellet was placed in the cuvette to which 5 μl of the drug solution was added for 3 mim preincubation at 37°C. Platelet aggregation was induced by collagen and ADP and the extent of aggregation was expressed taking the change in light transmission. PRP was used on the experiment of ADP or collagen induced aggregation and resuspension of the platelet pellet was used to experiment of PAF induced aggregation. The drug solutions were prepared in DMSO to give the final concentration of 0.3% by dilution of saline.

RESULTS AND DISCUSSION

Chemistry

Synthesis of aspirin derivatives initially showed poor yield when aspirin as stating material and DCC (*N,N'*-dicyclohexylcarbodiimide) were used. Their reaction products contained (3',4'-methylenedioxy)acetoxyphenol which formed by the methyl group transfer during the esterification. Therefore, aspirin derivatives were prepared from salicylic acid and phenolic compounds by esterification in the presence of 1,1-carbonydiimidazole followed by acetylation. (Fig. 1), Chemical structures of aspirin derivatives of SJ-101, 102, 103 and 104 were elucidated by analytical instruments.

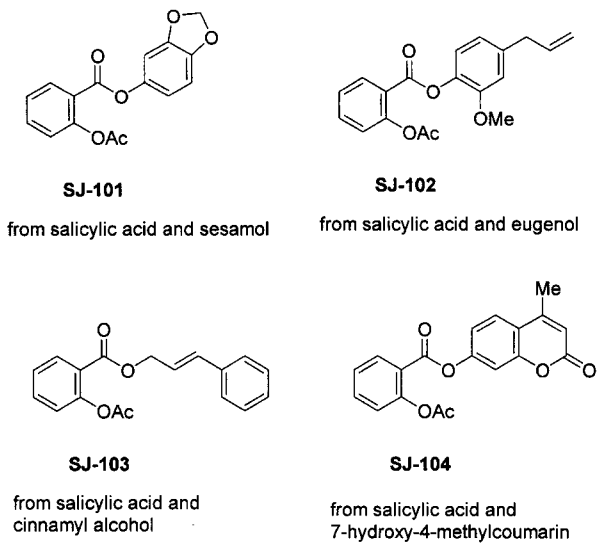

Fig. 1. Aspirin derivatives were prepared from salicylic acid and aromatic compounds in the presence of 1,1-carbonyldiimidazole followed by acetylation.

Antioxidant activity

Aspirin derivatives exhibited an antioxidant activity in *vitro* on Table I. SJ-101 was expressed to excellent inhibition effect of MDA(malondealdehyde) generation to comparable the value for BHA of synthetic antioxidant. This result mean that the only ester bond of SJ-101 was hydrolyzed by esterase from liver and exhibited effect of

Table I. Antioxidant effect of aspirin derivatives-inhibition % MDA generation in rat liver homogenate *in vitro* on TBA method

Treatment	Conc.(mol/l)	O.D(534 nm)	Inhibition(%)
Control	1.6×10^{-4}	2.240±0.051	
BHA	1.6×10^{-4}	0.576±0.108	74.29
Aspirin	1.6×10^{-4}	1.831±0.113	18.25
SJ-101	1.6×10^{-4}	0.337±0.030	84.95
SJ-102	1.6×10^{-4}	0.875±0.014	60.93
SJ-103	1.6×10^{-4}	1.668±0.013	25.54
SJ-104	1.6×10^{-4}	0.863±0.016	61.47

Values are means±S.E. from three separate experiments

Table II. Effect of aspirin derivatives on bleeding time in rats

Treatment	Dose (mg/kg)	No. of animals	Bleeding times (sec.)
Control	1 % CMC	5	323±90
Aspirin	20	5	521±54**
SJ-101	20	5	547±66**
SJ-102	20	5	255±20
SJ-103	20	5	468±65*
SJ-104	20	5	348±90

Value are mean±S.E. Bleeding time of adult male rats administrated orally (20 mg/kg/day)with various compounds for 10 days.
Significantly different with respect to control : *: $p<0.05$ **: $p<0.01$

Table III. Effect of SJ-101 on the platelet aggregation by some aggregation inducers

Treatment	Conc. (μg/ml)	% of inhibition		
		Collagen (10 μg/ml)	ADP(μM)	PAF(nM)
SJ-101	60			13.0
	30	100.0	11.5	0.0
	7.5	69.8		
	3.75	27.8		
	1.88	19.0		
Aspirin	30	78.8		
	20	61.0		
	10	45.1		
Adenosine	30		83.5	
	6		38.5	

antioxidant activity by sesamol.

Bleeding time prolongation

NSAID(non-steroidal anti-inflammatory drug) was known as a 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 NSAID. Therefore, synthesis of aspirin and salicylic acid derivatives followed by activity screenings was investigated (Casadebai *et al.*, 1991). Whereas the bleeding time of aspirin treated group was increased by 61% (521±54(s), n=5, $p<0.01$) compared to that of the control group (323±90, n=5), SJ-101 treatment prolonged the bleeding time by 69 % (547±66, n=5, $p < 0.01$) at the same dose of 20 mg/kg for 10 days (Table II). We thought 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 aspirin and free radical scavenging activity of sesamol and inhibition effect of prostaglandin cyclooxygenase of phenolic compounds (Dewhirst, 1980).

Anti-platelet aggregation activity

Because of SJ-101 was exhibited admirable antioxidant activity and effect of bleeding time prologation, we were investigated to the anti-platelet aggregation activity on SJ-101. As a result, SJ-101 showed a potent and concentration dependent inhibition of collagen-induced rabbit platelet aggregation. The IC_{50} value of SJ-101(4.8 μg/ml) was comparable to that of aspirin (12.3 μg/ml). But SJ-101 did not inhibit the first phase of ADP-induced and PAF-induced platelet aggregation (Table III).

ACKNOWLEDGEMENTS

This research was supported by the Sangji University Research Grants.

REFERENCES

Casadebaig, F., Dupin, J. P., Gravier, D., Hou, G., Daret, D., Bernard, H., Larrue, J. and Boisseau, M., Action of some salicylate derivatives on in vitro platelet aggregation inhibitory and inhibition antagonist effects. *Thromb. Res.*, 64, 631-636 (1991).

Dewhirst, F. E., Structure-activity relationships for inhibition of prostaglandin cyclooxygenase by phenolic compounds. *Prostaglandins*, 20, 209-222 (1980).

Flower, R., Gryglewski, R., Herbaczynska-cedro, K. and Vane, J. R., Effects of anti-inflammatory drugs on prostaglandin biosynthesis. *Nature New Bio.*, 238, 104-106 (1972).

Fukuzawa, K. and Takaishi, Y., Antioxidants. *J. Act. Oxyg. Free Rad.*, 1, 55-70 (1990).

Hatano, T., Constituents of natural medicines with scavenging effects on active oxygen species-Tannins and related polyphenols. *Natural Medicines*, 49, 357-363 (1995).

Hornstra, G., Christ-Hazelhof, E., Haddenman, E., Ten-Hoor, F. and Nugteren, D. H., Bleeding time, *Prostaglandins*, 21, 727-738 (1981).

Kitahara, K., Matsumoto, Y., Ueda, H. and Ueoka, R., A remarkable antioxidation effect of natural phenol derivatives on the autoxidation of -irradiated methyl linoleate. *Chem. Pharm. Bull.*, 40, 2208-2209 (1992).

Ko, E. N., Yeh, L. J., Liang, H. C., Kuo, S. C. and Teng, C. M., Mechanism of action of p-chlorobiphenyl on the inhibition of platelet aggregation. *J. Pharm. Pharmacol.*, 48, 395-400 (1996).

Masaki, H., Sakaki, S., Atsumi, T. and Sakurai, H., Active-oxygen scavenging activity of plant extracts. *Biol. Pharm. Bull.*, 18, 162-166 (1995).

Masugi, F. and Nakamura, T., Measurement of thiobar-

bituric acid value in liver homogenate solubilized with sodium dodecylsulphate and variation of the values affected by vitamin E and drugs. *Vitamin*, 51, 21-29 (1977).

Patrono, C., Aspirin and human platelets from clinical trials to acetylation of cyclooxygenase and back. *TiPS*, 10, 453-458 (1989).

Schoemaker, R. G., Saxena, P. R. and Kalkman, E. A., Low-dose aspirin improves *in vivo* hemodynamics in conscious, chronically infarcted rats. *Cardiovasc. Res.*, 37, 108-114 (1998).

Underwood, M. J. and More, R. S., Aspirin benefits patients with vascular disease and those undergoing revascularisation, *British Medical Journal*, 308, 71-72 (1994).

Vane, J. R., Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Bio.*, 231, 232-235 (1971).

Vane, J. R. and Botting, R., Inflammation and mechanism of action of anti-inflammatory drugs. *The FASEB Journal*, 1, 89-96 (1987).

Wallenburg, H. C., Dekker, G. A., Makovitz, J. W. and Rotmams, P., Low -dose aspirin prevents pregnancy-induced hypertension and pre-eclampsia in angiotensin-sensitive primigravidae. *Lancet*. 1, 1-3 (1986).