Cotonoates A and B, New Aromatic Esters from *Cotoneaster racemiflora*

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Phytochemical investigation of the chloroform soluble fraction of the methanolic extract of *Cotoneaster racemiflora* resulted in the isolation of two new aromatic esters named cotonoates A (**1**) and B (**2**) along with the known compound methyl 3,4-dihydroxy-5-methoxybenzoate (**3**). The structures of the new compounds have been assigned on the basis of spectral analysis including 1D and 2D NMR techniques. Compound **3** shows significant antioxidant and lipoxygenase inhibitory activities.

Key words: Cotoneaster racemiflora, Aromatic Ester, Cotonoate A, Cotonoate B

Introduction

The genus *Cotoneaster* belongs to the family Roseaceae which comprises 85 species, occurring mainly in Iran, Syria, Turkey, and Europe. It is represented in Pakistan by 9 species [1]. Various species of the genus *Cotoneaster* are used as cooling, laxative, aperient, astringent, and expectorant agents and also in the treatment of diseases of the eye, bronchitis, abdominal pain, stangury, thirst, itch, leucoderma, fevers, wounds, piles, and in urinary calculi [2 – 4]. *Cotoneaster racemiflora* Desf. occurs in the Western Himalayas from Kashmir to Kuman and from Siberia and Kabul to France. In Pakistan, it is very common in Baluchistan, Landi Kotal, Chitral, Swat, Kaghan, and Kashmir [5]. The plant has a bitter and sharp taste and is used medicinally in Indo-China. No phytochemical work has so far been carried out on this species.

The diverse medicinal applications of preparations from the genus *Cotoneaster* prompted us to carry out phytochemical investigations on *C. racemiflora*. The methanolic extract of the whole plant was subsequently divided into *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and water-soluble fractions. The chloroform-soluble fraction showed strong antioxidant activity. This fraction was, therefore, subjected to a series of column and flash chromatographic techniques. As a result two new aromatic esters named cotonoates A (**1**) and B (**2**) along with a known ester, methyl 3,4-dihydroxy-5-methoxybenzoate (**3**), have been isolated. Compound **3** showed significant antioxidant and lipoxygenase inhibitory activities.

Results and Discussion

Compound **1** was isolated as a colorless gummy solid and gave effervescence with dilute $NaHCO_3$ solution. The absorptions at 3260 – 2610, 1700 – 1720 and 1626 cm^{-1} in the IR spectrum showed the presence of carboxylic acid and ester groups and of a benzene ring. The molecular formula was determined through HREI-MS as $C_{17}H_{24}O_4$ (*m/z* = 292.3710; calcd. 292.3700) showing six degrees of unsaturation. The ^1H NMR spectrum of **1** showed a broad singlet appearing at δ = 11.92 confirming the presence of a carboxylic group. A pair of double doublets appearing at δ = 7.50 (2H, *J* = 8.4, 3.3 Hz) and 7.68 (2H, *J* = 8.4, 3.3 Hz) indicated a 1-4 disubstituted aromatic ring. A triplet at δ = 4.14 (2H, *J* = 5.7 Hz) was attributed to the oxymethylene protons. The appearance of a peak at *m/z* = 165 in EIMS was attributed to the loss of a C_9H_{19} alkyl chain. The oxymethylene protons showed ^1H-^1H COSY correlation with a methine proton at δ = 2.25. The latter in turn showed ^1H-^1H COSY correlations to the methyl protons at δ = 1.25 and the methylene protons at δ = 4.14 and δ = 1.42, respectively. This allowed us to place one of the methyl groups at the C-2′ position. The terminal methyl group appeared

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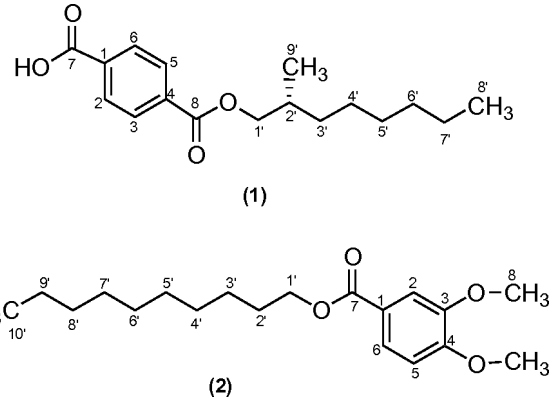
Table 1. ^{1}H and ^{13}C NMR data of **1** and **2** ($CDCl_3$, δ in ppm, J in Hz) and important HMBC correlations.

	1				**2**		
H/C	δ_H	δ_C	HMBC	H/C	δ_H	δ_C	HMBC
1	Q	126.9		1	Q	123.9	
2 (CH)	7.50 (dd; 8.8, 3.3)	128.7	2, 4, 6	2 (CH)	7.50 (d; 1.5)	114.0	4, 6, 7
3 (CH)	7.68 (dd; 8.8, 3.3)	130.8	1, 5, 8	3	Q	146.1	
4	Q	132.4		4	Q	150.0	
5 (CH)	7.68 (dd; 8.8, 3.3)	130.8	1, 3, 8	5 (CH)	6.90 (d; 8.2)	111.7	3, 1
6 (CH)	7.50 (dd; 8.8, 3.3)	128.7	2, 4, 7	6 (CH)	7.59 (dd; 8.2,1.5)	124.13	2, 4, 7
7	Q	177.3		7	Q	166.8	
8	Q	167.7		8 (CH_3)	3.85 (s)	56.0	3
1′ (CH_2)	4.14 (t; 5.7)	68.1	3′, 9′	9 (CH_3)	3.89 (s)	51.8	4
2′ (CH)	2.25 (m)	31.9	4′	1′ (CH_2)	4.14 (t; 5.7)	68.1	
3′ (CH_2)	1.42 (m)	34.7	1′, 5′, 9′	2′ (CH_2)	1.62 (m)	29.2	
4′ (CH_2)	1.25 (m)	27.4		3′ (CH_2)	1.41 (m)	23.7	
5′ (CH_2)	1.25 (m)	29.5		4′ (CH_2)	1.22 (br s)	29.6	
6′ (CH_2)	1.31 (m)	31.3		5′ (CH_2)	1.22 (br s)	29.6	
7′ (CH_2)	1.31 (m)	22.7		6′ (CH_2)	1.22 (br s)	29.6	
8′ (CH_3)	0.81 (d; 6.4)	14.5		7′ (CH_2)	1.22 (br s)	29.6	
9′ (CH_3)	1.25 (m)	19.2	1′, 3′	8′ (CH_2)	1.22 (br s)	31.8	
				9′ (CH_2)	1.31 (m)	22.6	
				10′ (CH_3)	0.82 (t; 6.7)	14.0	

at δ = 0.81 (t, J = 6.6 Hz). The remaining four methylene protons resonated between δ = 1.25 – 1.31.

The fifteen carbon signals in the ^{13}C NMR (BB) spectrum were resolved through a DEPT experiment into two methyl, six methylene, three methine, and four quaternary carbon signals (Table 1). The most downfield signals at δ = 177.3 and δ = 167.7 were assigned to the acid and ester carbonyls, respectively. Other signals appearing at δ = 132.4 and δ = 126.9 were assigned to C-4 and C-1, respectively. The carbon signal appearing at δ = 68.1 was attributed to the oxymethylene carbon. The entire chemical shifts were confirmed through HMQC and HMBC experiments. The stereochemistry at the chiral center C-2′ was determined through the alkaline hydrolysis of **1** that provided an alcohol which could be identified as (*R*)-2-methyl-1-octanol through the sign of its optical rotation. On the basis of these evidences the structure of cotonoate A (**1**) was assigned as 4-({[(2*R*)-2-methyloctyl]oxy}carbonyl) benzoic acid (Fig. 1).

Compound **2** was also isolated as a colorless gummy solid. The IR spectrum showed absorption bands at 1710 and 1626 cm^{-1} indicative of ester and aromatic groups. The molecular formula was determined as $C_{19}H_{30}O_4$ through HREI-MS (*m/z* = 322.4381; calcd. 322.4391) showing five degrees of unsaturation. The ^{1}H NMR spectrum showed a trisubstituted benzene moiety [two doublets appearing at δ = 7.50 (1H, J = 1.5 Hz, H-2) and δ = 6.9 (1H, J = 8.2 Hz, H-5), and a double doublet appearing at δ = 7.59 (1H, J = 8.2 and 1.5 Hz, H-6)]. The oxymethyl protons appeared as two singlets at δ = 3.85 and δ = 3.89 while the oxymethylene protons appeared at δ = 4.19 (t, J = 5.7 Hz). The terminal methyl group gave a triplet at δ = 0.82 (t, J = 6.7 Hz) while the signals appearing between δ = 1.62 and δ = 1.25 integrated for sixteen protons. The presence of a $C_{10}H_{21}$ side chain was also confirmed by EI-MS which gave an intense peak at *m/z* = 181 due to the loss of 141 mass units.



(1)

(2)

Fig. 1. Structures of cotonoates A (**1**) and B (**2**).

The sixteen carbon signals in the ^{13}C NMR (BB) spectrum were resolved through a DEPT experiment into three methyl, six methylene, three methine, and four quaternary carbon signals (Table 1). The most downfield-shifted signal at δ = 166.8 was assigned to the carbonyl carbon. Other quaternary carbons, appear-

Table 2. Antioxidant and lipoxygenase inhibition activities of compounds **1**, **2** and **3**[a].

Compound	DPPH assay (IC_{50})	Lipoxygenase inhibition (IC_{50})
1	NS	NS
2	NS	NS
3	9.7 ± 0.12	19.5 ± 0.07
BHA[b]	44.3 ± 0.09	
Baicalein[b]		22.6 ± 0.08

[a] Results are expressed as IC_{50} values (μM); data for active compounds are the mean of triplicates; NS = not significant; [b] positive control.

ing at δ = 123.9, 146.1 and 150.0, were assigned to C-1, C-3 and C-4, respectively. The signal appearing at δ = 68.1 was attributed to the oxymethylene carbon. The chemical shifts were assigned through HMQC and HMBC experiments. The structure of cotonoate B (**2**) was thus assigned as decyl-3,4-dimethoxy benzoate (Fig. 1).

Compound **3** was obtained as a crystalline yellow solid. The IR spectrum showed the presence of carbonyl (1694 cm^{-1}) and hydroxyl groups (3319 and 3467 cm^{-1}). Comparison of the ^1H and ^{13}C NMR data with those reported in the literature identified **3** as methyl-3,4-dihydroxy-5-methoxybenzoate [6].

For the screening and evaluation of any antioxidant activity of the isolated compounds a DPPH scavenging assay was adopted. Baicalein was used as positive control in the lipoxygenase inhibiting assay. Only compound **3** exhibited profound antioxidant activity and significant inhibitory activity against lipoxygenase enzymes (Table 2). These compounds were also screened against chymotrypsine, urease, and cholinesterase, but no significant activity could be observed.

Experimental Section

General

TLC: Precoated Aluminium sheets with silica gel 60 F_{254} 20×20 cm^2, 0.2 mm thick, from Merck, Darmstadt, Germany. Column chromatography (CC): silica gel (70 – 230 and 230 – 400 mesh) from Merck, Darmstadt, Germany. Optical rotations: Jasco DIP-360 digital polarimeter. IR spectra: Shimadzu IR-460 spectrophotometer; KBr pellets. NMR spectra: Bruker AMX-400 MHz instrument; $SiMe_4$ as an internal standard; δ in ppm, J in Hz. MS: Jeol JMS-HX 110 spectrometer.

Plant material

The whole plant of *Cotoneaster racemiflora* Desf. was collected from Swat (Pakistan) in July 2004 and identified by Prof. Dr. Jahndar Shah, Plant Taxonomist, Islamia College Peshawar, University of Peshawar, where a voucher specimen (CR-119-04) is deposited.

Extraction and isolation

The shade dried whole plant of *C. racemiflora* (15 kg) was extracted with MeOH (3×50 L) at r. t. The combined methanolic extract was evaporated under reduced pressure to obtain a thick gummy residue (500 g). This residue was suspended in water and successively extracted with *n*-hexane (49 g), chloroform (210 g), ethyl acetate (40 g), and *n*-butanol (60 g). The chloroform-soluble fraction was subjected to column chromatography over silica gel eluting with *n*-hexane, *n*-hexane-chloroform, chloroform, chloroform-methanol, and methanol in increasing order of polarity.

The fraction obtained from *n*-hexane-chloroform (4.0 : 6.0) showed a major spot on TLC. It was resubjected to flash chromatography eluting with the solvent system *n*-hexane-chloroform (3.0 : 7.0) to afford cotonoate A (**1**) (22 mg).

Cotonoate B (**2**) was obtained in semi-pure form from the column using *n*-hexane-chloroform (4.0 : 6.0) as eluent. Further purification by flash chromatography with *n*-hexane-chloroform (5.0 : 5.0) as an eluent afforded the pure compound (18 mg).

Compound **3** was purified (20 mg) by column chromatography using hexane/chloroform (2.0 : 8.0) as solvent.

*Cotonoate A (**1**)*

Gummy solid. – $[\alpha]_D^{25}$ = +44 (c = 0.12, MeOH). – IR (KBr): ν = 3260 – 2610 (COOH), 1700 – 1720 (carbonyl), 1626 (aromatic) cm^{-1}. – ^1H NMR, ^{13}C NMR and HMBC correlations see Table 1. – HREIMS: m/z = 292.3710 (calcd. 292.3700 for $C_{17}H_{24}O_4$, $[M]^+$).

*Acid hydrolysis of **1***

To a solution of the ester **1** (20 mg) in a CH_2Cl_2/CH_3OH (0.9 : 0.1; 1 mL) mixture was added a methanolic solution of NaOH (0.05 mL; 3 N). After 2 – 3 min of stirring, the solution became cloudy, and the sodium salt of the acid precipitated. Stirring was continued for 30 min. The solvent was then removed *in vacuo*, the residue was diluted with water, and the aqueous layer was extracted with ethyl acetate. Removal of the solvent afforded the alcohol (8.8 mg) as a viscous oil. The sign of optical rotation $[\alpha]_D^{22}$ = +15.7 (c = 0.16; EtOH) and spectral data identified it as (*R*)-2-methyl-1-octanol [7]. The aqueous layer was then cooled and acidified with dilute HCl and extracted with ethyl acetate. The organic layer was evaporated *in vacuo* to afford the free acid (10 mg). It could be identified as terephthalic acid by comparison with an authentic sample (m. p. 304 °C).

*Cotonoate B (**2**)*

Gummy solid. – IR (KBr): ν = 1710 (ester), 1626 (aromatic) cm^{-1}. – ^1H NMR, ^{13}C NMR and HMBC correlations see Table 1. – HREIMS: *m/z* = 322.4381 (calcd. 322.4391 for $C_{19}H_{30}O_4$, $[M]^+$).

Antioxidant assay

The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method described by Gulcin *et al.* [8]. A 0.3 μM solution of DPPH was prepared in ethanol. Five μL of each sample of different concentration (62.5 – 500 μg) was mixed with 95 μL of a DPPH solution in ethanol. The mixture was dispersed in a 96 well plate and incubated at 37 °C for 30 min. The absorbance at 515 nm was measured by a microtitre plate reader (Spectramax plus 384 Molecular Device, USA), and percent radical scavenging activity was determined in comparison with the methanol-treated control. BHA was used as standard. – DPPH scavenging effect (%) = $(Ac - As) \times 100$, where Ac = Absorbance of control (DMSO treated), As = Absorbance of sample.

Lipoxygenase inhibition assay

Lipoxygenase inhibiting activity was measured by modifying the spectrophotometric method developed by Tappel [9]. A lipoxygenase enzyme solution was prepared so that the enzyme concentration in the reaction mixture was adjusted to give rates of 0.05 absorbance min^{-1}. The reaction mixture contained 160 μL (100 mm) of a sodium phosphate buffer (pH = 8), 10 μL of the test solution and 20 μL of the LOX solution. The contents were mixed and incubated for 10 min at 25 °C. The reaction was then initiated by the addition of 10 μL substrate solution (linoleic acid, 0.5 μm, 0.12 % w/v tween 20, in ratio of 1 : 2) with the formation of (9*Z*,11*E*)-(13*S*)-13 hydroperoxyoctadeca-9,11-dienoate, and the change in absorbance at 234 nm was followed for 6 min. The concentration of the test compound that inhibited lipoxygenase activity by 50 % (IC_{50}) was determined by monitoring the effect of increasing concentrations of these compounds in the assays on the degree of inhibition. The IC_{50} values were calculated by means of the EZ-fit, [enzyme kinetics program (Perrella Scientific Inc., Amherst, USA)].

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[1] S. I. Ali, Y. J. Nasir, *Flora of Pakistan*, Fakhri printing press, Karachi, **1989**, *191*, pp. 94.
[2] R. N. Sastri, *The Wealth of India*, CSIR India, **1950**, *2*, pp. 362.
[3] K. S. Mhaskar, E. Blatter, J. F. Caius (Eds.), *Kirtikar and Basu's illustrated Indian medicinal plants: Their usage in Ayurveda and Unani Medicines*, (3rd ed.), Vol. 5, Sri Satguru Publications, Indian books centre, Dehli, **2000**, pp. 1382 – 1383.
[4] L. M. Perry, J. Metzger, *Medicinal Plants of East and Southeast Asia*, MIT Press, Cambridge, **1980**, pp. 347.
[5] B. N. Meyer, N. R Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nicholas, J. L. McLaughlin, *Planta Med.* **1982**, *45*, 31 – 34.
[6] B. Meera, R. Singh, S. B. Kalidhar, *J. Indian Chem. Soc*. **2004**, *81*, 436 – 437.
[7] K. Mori, *Tetrahedron;Asymmetry*, **2008**, *19*, 857 – 861.
[8] I. Gulcin, H. A. Alici, M. Cesur, *Chem. Pharm. Bull.* **2005**, *53*, 281 – 285.
[9] A. L. Tappel in *Methods of Enzymology*, Vol. 5 (Eds.: S. P. Colowick, N. O. Kaplan), Academic Press, New York, **1962**, pp. 539 – 542.