Communications to the Editor

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ISOLATION AND STRUCTURAL ELUCIDATION OF A NEW LIPOXYGENASE INHIBITOR FROM GARDENIAE FRUCTUS

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A new lipoxygenase inhibitor, 3,4-dicaffeoyl-5-(3-hydroxy-3-methylglutaroyl)quinic acid (1), has been isolated from Gardeniae Fructus. The structure of 1 was determined from its spectral data and by methanolysis of its methylate (2).

KEYWORDS———Gardeniae Fructus; *Gardenia jasminoides*; Rubiaceae; lipoxygenase inhibitor; quinic acid; caffeic acid; 3-hydroxy-3-methylglutaric acid

Gardeniae Fructus (fruit of *Gardenia jasminoides* ELLIS, Rubiaceae) is a crude drug which has been used as an antiphlogistic, a diuretic and a chalagogues in China and Japan. The constituents of *Gardenia* sp. have been studied since the eighteenth century, and a number of iridoid glycosides[2,3,4] and yellow pigments[5,6] have been isolated from it.

In this report, we describe the isolation and structural elucidation of a new lipoxygenase inhibitor from Gardeniae Fructus collected in China.

The powdered Gardeniae Fructus was extracted with n-hexane to remove paraffins, fatty acids and triglycerides, and then the residue was extracted three times with 50% aq. acetone. After evaporation of the acetone under reduced pressure, the solution was extracted with ethyl acetate and then with n-butanol.

The new compound (1) [amorphous powder, $C_{31}H_{32}O_{16}$, $[\alpha]_D^{18}$ -169.8° (c = 0.95, MeOH), λmax(MeOH) : 329, 242 and 216 nm (log ε 4.49, 4.25 and 4.42), νmax(KBr) : 3200-3400(OH), 1710-1680(COO) cm^{-1}, NMR data: see note 7], together with some known compounds (geniposide[2], genipin gentiobioside[3], crocin[5] and rutin), was isolated from the n-butanol extracts by column chromatography (HP-20, MeOH-H_2O gradient) and then HPLC (ODS, MeOH-H_2O-AcOH).

The compound (1) showed the ion peaks at m/z 683 (M^+ + Na) and at m/z 661(M^+ + 1) in its FD-MS spectrum. Treatment of 1 with diazomethane gave a mixture of several methylates which was further methylated with dimethyl sulfate-potassium carbonate to give a hexamethylate (2) [MS: m/z 744(M^+), NMR data: see note 8]. The ^{1}H-NMR spectrum of 1 showed two pairs of the signals at δ 7.62, 6.33 (doublet each, J = 15.9 Hz), and at δ 7.56, 6.25 (doublet each, J = 15.9 Hz) due to *trans* olefins and a pair of signal due to 1,3,4-trisubstituted aromatic protons. This

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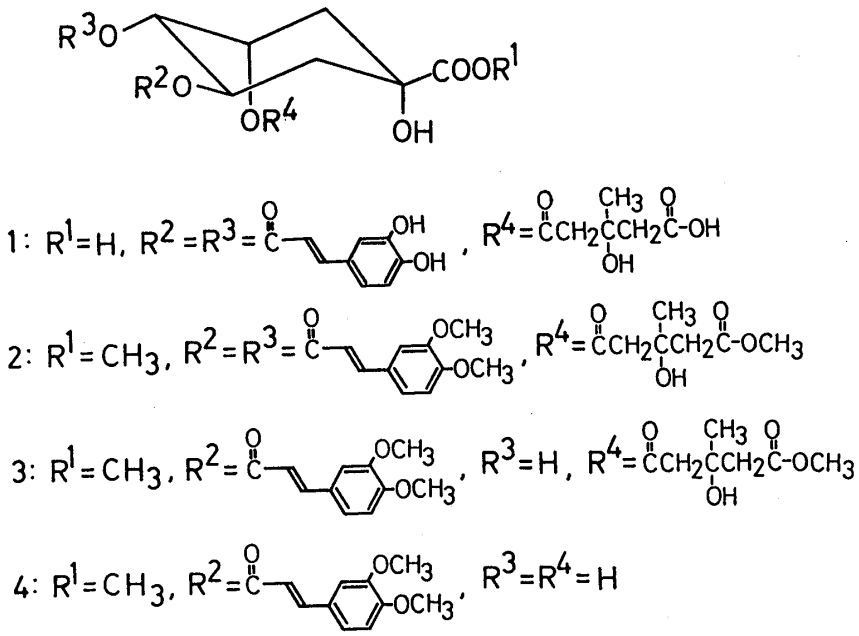
suggests the presence of 2 moles of a caffeic acid moiety in the molecule. In addition, the decoupling experiments on 2 indicated that 2 was a 3,4,5-acylated quinic acid derivative.



Chart

Treatment of 2 with 5% MeOH-HCl gave a decaffeoyl derivative (3) [MS: m/z 586(M^+), NMR data: see note 9] , dimethylchlorogenic acid methyl ester (4), dimethylcaffeic acid methyl ester (5) and 3-methyl-3-hydroxyglutaric acid dimethyl ester (6). The structure of 3 was confirmed as the 4-decaffeoyl derivative of 2, because the H-4 (δ 3.80 ppm) of 3 resonated at the 1.46 ppm high field as compared with that (δ 5.26 ppm) of 2. The compounds (4, 5, 6) were identified with their authentic samples derived from corresponding carboxylic acids[10] by the usual means.

From the detailed ^{1}H-NMR decoupling experiments on 2 and 3 and the results of methanolysis of 2, the structure of 1 could be confirmed as 3,4-dicaffeoyl-5-(3-hydroxy-3-methylglutaroyl)quinic acid. The compound (1) inhibited the activities of 5- and 12-lipoxygenase, 92% and 93% at 100 μM, respectively. The biological activities of 1 and of its relatives will be published elsewhere.

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7) ^{1}H-NMR(400 MHz, acetone-d_6, δ): 1.37(3H, s), 2.20-2.52 (3H, m), 2.70-2.77(4H, m), 5.35(1H, m), 6.25(1H, d, J=15.9 Hz), 6.34(1H, d, J=15.9 Hz), 6.90(2H, m), 7.05(1H, m), 7.20(2H, m), 7.57(1H, d, J=15.9 Hz), 7.63(1H, d, J=15.9 Hz). ^{13}C-NMR(22.5 MHz, acetone-d_6, δ) : 27.1, 35.8, 37.8, 44.6, 45.7, 67.9, 68.7, 69.5, 72.3, 73.6, *114.7, *115.1, *115.8, *122.1, 127.1, 127.2, *145.6, 145.7, 145.9, *148.1, 165.9, 166.3, 170.4, 172.6, 175.0. (* two carbons)

8) ^{1}H-NMR(400 MHz, $CDCl_3$, δ) : 1.30(3H, s; GA-CH_3), 2.14(1H, dd, J=10.9, 13.1 Hz; QA-C2-H axial), 2.24(1H, br d, J=15.1 Hz; QA-C6-H equatorial), 2.33(1H, dd, J=3.4, 15.1 Hz; QA-C6-H axial), 2.43(1H, br d, J=13.1 Hz; QA-C2-H equatorial), 2.59(2H, m; GA-CH_2), 2.66(2H, m; GA-CH_2), 3.59(3H, s; $COOCH_3$), 3.82(3H, s; $COOCH_3$), 3.92(3H, s; OCH_3), 3.925(3H, s; OCH_3), 3.93(3H, s; OCH_3), 3.96(3H, s; OCH_3),5.26(1H, dd, J=3.4, 9.8 Hz; QA-C4-H), 5.66(1H, m; QA-C5-H), 5.72(1H, m; QA-C3-H), 6.25(1H, d, J=15.9 Hz; CA-olefin), 6.37(1H, d, J=15.7 Hz; CA-olefin), 6.87(1H, d, J=6.9 Hz; CA-C2-H), 6.88(1H, d, J=6.9 Hz; CA-C2-H), 7.07(1H, dd, J=2.0, 6.9 Hz; CA-C6-H), 7.11(1H, d, J=2.0 Hz; CA-C4-H), 7.13(1H, dd, J=2.0, 6.9 Hz; CA-C6-H), 7.71(1H, d, J=16.9 Hz; CA-olefin), 7.79(1H, d, J=15.9 Hz; CA-olefin). ^{13}C-NMR(22.5 MHz, $CDCl_3$, δ) : 27.3(GA-CH_3), 36.6(QA-CH_2), 44.7(GA-CH_2), 45.3(GA-CH_2), 51.6($COOCH_3$), 53.2($COOCH_3$), 56.1(OCH_3 X 4), 67.3(QA-CH), 68.7(QA-CH), 69.6(GA-C-3), 72.3(QA-CH), 74.2(QA-C-1), *110.0(CA), *112.3(CA), 115.2(CA), 122.8(CA), 123.2(CA), 127.3(CA), 145.7(CA), 145.9(CA), *149.5(CA), *151.5(CA), 166.0(COO), 166.3(COO), 170.8(COO), 172.1(COO), 174.5(COO). CA, QA and GA in the data represent caffeic acid, quinic acid and 3-hydroxy-3-methylglutaric acid moieties. (* two carbons).

9) ^{1}H-NMR(400 MHz, $CDCl_3$, δ) : 1.42(3H, s, GA-CH_3),2.03(1H, dd, J=11.0, 12.2 Hz; QA-C2-H), 2.23(2H, m; QA-C6-H), 2.31(1H, m; QA-C2-H), 2.66-2.78(4H, m; GA-CH_2), 3.73(3H, s; $COOCH_3$), 3.79(3H, s; $COOOCH_3$), 3.917(3H, s; OCH_3), 3.921(3H, s; OCH_3), 3.80(1H, m; QA-C4-H), 5.47(1H, m; QA-C5-H), 5.50(1H, m; QA-C3-H), 6.32(1H, d, J=15.9 Hz; CA-olefin), 6.87(1H, d, J=8.3 Hz; CA-C5-H), 7.05(1H, d, J=1.7 Hz; CA-C2-H), 7.11(1H, dd, J=1.7, 8.3 Hz; CA-C6-H), 7.65(1H, d, J=15.9 Hz; CA-olefin).

10) Caffeic acid, 3-hydroxy-3-methylglutaric acid and chlorogenic acid were obtained from Tokyo Kasei Co., Ltd.

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