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PHYTOTOXIC CONSTITUENTS OF THE FRUITS OF *Averrhoa carambola*

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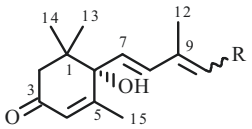
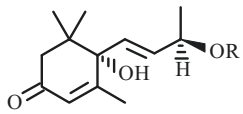
The agrochemical industry is continuously searching for phytotoxic compounds, including weedicides, herbicides, etc. The continuous use of synthetic phytotoxic compounds may affect nontarget organisms and also cause environmental pollution problems due to retarded degradation, etc. Therefore it is important to search for natural phytotoxic compounds to replace or to reduce the use of synthetic hazardous phytotoxic compounds. In a continuation of our search for biologically active compounds from edible fruits of Sri Lanka, recently we investigated the fruit of *Averrhoa carambola* L. (star fruit) of the family Oxalidaceae. *A. carambola* is a slow growing, moderate sized shrub-like ornamental tree distributed in Asian countries. Various parts of *A. carambola* have been used for the treatment of vomiting, internal bleeding piles, asthma, colic, jaundice, and skin diseases [1]. It is also used topically on poisonous bites and stings. A variety of carotenoid-derived compounds has been reported from star fruit [2]. It has been reported that star fruit is a very good source of natural antioxidants and ascorbic acid; epicatechin and gallic acid derivatives were suggested as the main antioxidants [3]. In this paper we report the isolation and structure elucidation of nine compounds (**1–9**) from the fruit of *A. carambola* and their phytotoxicity in lettuce seed germination.

Fresh ripe fruits (6 kg) of *A. carambola*, collected from the central province of Sri Lanka, were homogenized using a domestic blender, and the homogenate was filtered through a Buchner funnel. The light green colored juice was extracted with EtOAc and then BuOH. Evaporation of EtOAc gave a brown solid (3.70 g). The filter cake was extracted with MeOH, and the extract was concentrated and partitioned between BuOH and H_2O. Both BuOH extracts from the juice and the filter cake were combined and concentrated to give a dark brown solid (18.7 g). Preliminary phytotoxic assay indicated that both EtOAc and BuOH extracts were active. Therefore, both extracts were combined and subjected to a combination of chromatography on silica gel (*n*-hexane–EtOAc–MeOH), Sephadex LH-20 (MeOH), and reverse phase silica gel (H_2O–MeOH) and reverse phase HPLC (solvent, 50–65% aqueous MeOH) to yield *cis*-abscisic acid (**1**, 12 mg) [4], *trans*-abscisic acid (**2**, 3.5 mg) [5], *trans*-abscisic alcohol (**3**, 12 mg) [6], (6*S*,9*R*)-vomifoliol (blumenol A), (**4**, 8.5 mg) [7], *cis*-abscisic acid β-D-glucopyranosyl ester (**5**, 165 mg) [8], *trans*-abscisic alcohol β-D-glucopyranoside (**6**, 19 mg) [9], (6*S*,9*R*)-roseoside (**7**, 12 mg [10], *cis*-abscisic alcohol β-D-glucopyranoside (**8**, 12 mg) and (–)-epicatechin (**9**, 113 mg) [11]. These compounds except for **6** and **8** were identified by spectral comparison with the reported data.



1 - 3, 5, 6, 8



4, 7

1: R = *cis*-COOH; **2:** R = *trans*-COOH; **3:** R = *trans*-CH_2OH
4: R = H; **5:** R = *cis*-COOGlc; **6:** R = *trans*-CH_2OGlc
7: R = Glc; **8:** R = *cis*-CH_2OGlc

Compound **6** was isolated for the first time, although its tetra-acetate derivative was reported previously [9]. The spectral data for **6** is therefore described here. Compound **6**: colorless sticky solid. UV (MeOH, λ_{max}, nm): 234.

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^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 1.00 (3H, s, H-13), 1.04 (3H, s, H-14), 1.82 (3H, s, H-12), 1.90 (3H, s, H-15), 2.17 (1H, d, J = 16.9, H_a-2), 2.48 (1H, d, J = 16.9, H_b-2), 3.17 (1H, dd, J = 8.5, 7.9, H-2′), 3.22–3.35 (3H, m, H-3′, 4′, 5′), 3.66 (1H, dd, J = 11.9, 5.4, H_a-6′), 3.86 (1H, br.d, J = 11.9, H_b-6′), 4.28 (1H, d, J = 7.9, H-1′), 4.36 (1H, dd, J = 12.8, 7.3, H_a-11), 4.50 (1H, dd, J = 12.8, 6.3, H_b-11), 5.70 (1H, dd, J = 7.3, 6.3, H-10), 5.82 (1H, d, J = 15.9, H-7), 5.89 (1H, s, H-4), 6.36 (1H, d, J = 15.9, H-8). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 42.7 (C-1), 50.8 (C-2), 201.2 (C-3), 127.2 (C-4), 167.3 (C-5), 80.4 (C-6), 130.0 (C-7), 135.8 (C-8), 137.5 (C-9), 129.4 (C-10), 66.4 (C-11), 13.0 (C-12), 23.5 (C-13), 24.6 (C-14), 19.6 (C-15), 103.3 (C-1′), 75.1 (C-2′), 78.0 (C-3′), 71.7 (C-4′), 78.1 (C-5′), 62.8 (C-6′). FAB-MS *m/z* 435 $[M + Na]^+$.

Compound **8** was obtained in trace amounts from a transgenic mutant of *Nicotiana plumbaginifolia* previously, and its structure was assigned tentatively on the basis of MS data without any evidence for the geometry of the double bond [12]. We describe here the NMR data of **8** for the first time, which were assigned by 2D NMR studies including HMBC. The *cis* geometry for the C-9/C-10 double bond was established by NOE experiments. Compound **8**: amorphous solid; $[\alpha]_D^{25}$+128° (*c*, 0.17, MeOH). UV (MeOH, λ_{max}, nm): 234. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 1.00 (3H, s, H-13), 1.05 (3H, s, H-14), 1.88 (3H, s, H-12), 1.91 (3H, s, H-15), 2.20 (1H, d, J = 17.0, H-2a), 2.51 (1H, d, J = 17.0, H_b-2), 3.16 (1H, dd, J = 8.4, 7.9, H-2′), 3.22–3.35 (3H, m, H-3′, 4′, 5′), 3.68 (1H, dd, J = 11.9, 5.6, H_a-6′), 3.89 (1H, br.d, J = 11.9, H_b-6′), 4.28 (1H, d, J = 7.9, H-1′), 4.36 (1H, dd, J = 12.5, 7.7, H_a-11), 4.49 (1H, dd, J = 12.5, 6.5, H_b-11), 5.61 (1H, dd, J = 7.7, 6.5, H-10), 5.88 (1H, s, H-4), 5.90 (1H, d, J = 15.9, H-7), 6.80 (1H, d, J = 15.9, H-8). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 42.6 (C-1), 50.7 (C-2), 201.1 (C-3), 127.1 (C-4), 167.1 (C-5), 80.5 (C-6), 132.5 (C-7), 128.2 (C-8), 136.7 (C-9), 127.2 (C-10), 65.4 (C-11), 20.7 (C-12), 23.6 (C-13), 24.8 (C-14), 19.5 (C-15), 103.2 (C-1′), 75.1 (C-2′), 78.0 (C-3′), 71.6 (C-4′), 78.1 (C-5′), 62.8 (C-6′). HR-FAB-MS(+) *m/z* 435.1970 $[M + Na]^+$ ($C_{21}H_{32}O_8Na$ requires 435.1995).

Compounds **3–9** were subjected to phytotoxic bioassay against lettuce (*Lactuca sativa*) seed germination using a petri dish method [13]. The compounds were tested at concentrations ranging from 5 to 1000 ppm, and germination was observed daily for five days. (±)-Abscisic acid (purchased from Sigma– Aldrich) was used as a positive control [14, 15].

The phytotoxic activities of **3–9** are expressed as IC_{50} values: (±)-abscisic acid (5 ppm), **3** (52 ppm), **4** (10 ppm), **5** (5 ppm), **6** (80 ppm), **7** (>500 ppm), **8** (10 ppm), and **9** (>500 ppm). *cis*-Abscisic acid glucoside (**5**) showed the most potent inhibitory activity comparable to (±)-abscisic acid, in accordance with the previous report [8]. *cis*-Abscisic alcohol glucoside (**8**) and vomifoliol (**4**) showed 50% activity against the control. This is the first report to demonstrate the phytotoxic activities of the contents of the fruit of *A. carambola*. None of compounds **3**–**8** showed antioxidant activity in an assay examining the radical scavenging effect toward DPPH radicals, although the antioxidant activity of (–)-epicatechin (**9**) was confirmed in this study. This study provides evidence that edible fruits are a potential source of environmental friendly bioactive compounds since their safety and toxicological issues related to human beings are remarkably less than those of other natural sources.

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