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ETHYL BREVIFOLIN CARBOXYLATE AND OTHER CONSTITUENTS FROM *ACER OBLONGUM* LEAVES

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Key Word Index—*Acer oblongum*; Aceraceae; 1,2,3,5-tetrahydro-7,8,9-trihydroxy-3,5-dioxocyclopenta[c] [2] benzopyran-1-carboxylic acid ethyl ester; ethyl brevifolin carboxylate; D-3-*O*-methyl *chiro*inositol; structural determination.

Abstract—A new compound, ethyl brevifolin carboxylate, along with known compounds D-3-*O*-methyl *chiro*inositol, β-amyrin, β-sitosterol, apigenin, kaempferol, ethyl gallate and quercetin were isolated from *Acer oblongum* and indentified from spectral and chemical data.

INTRODUCTION

Acer is a large genus of trees and a few shrubs, chiefly distributed in the north temperate region. About 15 species of *Acer* occur in the Himalayas [1]. Kupchan *et al.* have isolated a novel triterpene from the tumour inhibitory saponin of *A. negundo* [2]; the plant also contains novel glycosides [3–5]. Two antibacterial glycosides have been isolated from *A. ginnala* [6].

Since no work has been reported on *A. oblongum*, we now report the isolation and characterization of a new tannin, ethyl brevifolin carboxylate (**1**) along with other known compounds from this plant.

RESULT AND DISCUSSION

Extensive chromatography of the petrol extract of air-dried and powdered leaves of *A. oblongum* yielded β-sitosterol and β-amyrin [7]. The ethyl acetate extract on CC followed by prep. TLC gave a new compound along with apigenin, kaempferol, ethyl gallate and quercetin.

The new compound 1 gave a positive colour with ferric chloride reagent and its mass spectrum showed a $[M]^+$ at m/z 320 ($C_{15}H_{12}O_8$) and a base peak at m/z 274 $[M-MeCH_2OH]^+$. The IR spectrum exhibited a broad band between 3400–3000 cm^1 indicative of phenolic hydroxyls. IR bands appearing at 1695 and 1660 cm^{-1} are attributable to the α,β-unsaturated carbonyl group and the carbonyl of a lactone moeity, respectively, Another carbonyl appeared at 1735 cm^{-1}. The ^1H NMR spectrum (DMSO−d_6) of **1** showed a triplet at δ 1.18 ($J=7.3$ Hz, 3H) for an ester Me group, a quartet at δ 4.08 ($J=7.3$ Hz, 2H) for a methylene proton and a doublet of doublet for one hydrogen at δ 4.40 ($J=2$ and 7.7 Hz). The aromatic hydrogen appeared as a singlet at δ 7.30. The ^{13}C NMR spectrum of **1** showed a singlet at δ 13.813 for a methyl carbon and at δ 37.037 for a methylene carbon. The signal for the CH and CH_2 carbon of the five-membered ring appeared at δ 48.665 and δ 60.473, respectively, and for the aromatic CH at δ 108.318. The signals for other aromatic and sp^2 carbons appeared at δ 113.114, 114.951, 130.387, 140.148, 143.518, 145.749, 149.711, 160.048, 171.887 and 192.789.

Compound **1** on treatment with 10% HCl gave a yellow precipitate, mp > 245° which was comparable with that of an authentic sample of brevifolin carboxylic acid [8–10]. Compound **1** was methylated with diazomethane to give a trimethyl ether, mp 136°. It was comparable with the reported trimethyl ether of brevifolin carboxylic acid ethyl ester [11]. Compound **1** is, therefore, characterized as 1,2,3,5-tetrahydro-7,8,9-trihydroxy-3,5-dioxocyclopenta[*c*][2]benzopyran-1-carboxylic acid ethyl ester. This constitutes the first report of compound **1** from nature.

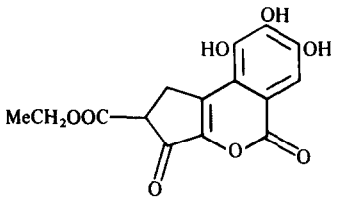
Compound **2**, $C_7H_{14}O_6[M+1]^+$ m/z 195 (100), showed a broad band in IR for OH at 3300 cm^{-1} and had $[\alpha]_D^{H_2O}+60$. The ^1H NMR spectrum (DMSO-d_6) of **2** showed a singlet at δ 3.35 assignable to the proton of the methoxy group. The six CH protons appeared between δ 3.25–3.9. The hydroxyl protons which were exchangeable with D_2O appeared at 4.26, 4.31, 4.43 (*q*) and 4.63 (*q*). In the ^{13}C NMR spectrum (DMSO−d_6) the methylene carbon appeared at δ 56.937 (*q*) and other carbons appeared as doublets at δ 68.002, 70.476, , 71.903, 72.131, 73.27 and 80.18. These data compared well with those of D-3-*O*-methyl *chiro*inositol [12]. The difference in chemical shifts between literature data and compound **2** may be due to the difference in the solvent used in measurements.

The details about the isolation and characterization of commonly occurring β-amyrin, β-sitosterol, ethyl gallate, apigenin, kaempferol and quercetin are given in Experimental.

EXPERIMENTAL

Mps: uncorr. NMR [δ(ppm), *J*(Hz)] were recorded using TMS as int. std. MS were determined at 70 eV.

Extraction and separation. Dried and powdered leaves of *A. oblongum* (2 kg) collected from the Royal Botanical Garden, Godawari, Lalitpur. Nepal were extd several times with hot



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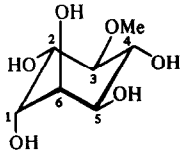

2

MeOH. The MeOH ext was evapd under red. pres. to yield a dark green mass. This was treated with petrol (60–80°), C_6H_6, EtOAc and then with MeOH.

The petrol and C_6H_6 fraction was chromatographed on a silica gel column and eluted with petrol–C_6H_6 (1:1) and benzene. The compound eluted with petrol–C_6H_6 (1:1) crystallized from $CHCl_3$–EtOH as colourless needles, mp 198°, and gave a violet red Liebermann–Burchard reaction. It was comparable (IR, MS, ^{1}H NMR) with an authentic sample of β-amyrin [7]. Another compound was eluted with C_6H_6, it crystallized from $CHCl_3$–EtOH as colourless needles, mp 137° and was found to be comparable (mmp, IR, ^{1}H NMR, MS) with β-sitosterol.

The EtOAc fraction gave an orange colour with Mg–HCl. The fraction was adsorbed on silica gel and transferred to a column of silica gel prepared with C_6H_6 and eluted with C_6H_6, C_6H_6–EtOAc (9:1, 4:1, 1:1) and EtOAc, mp 346°. It was comparable (mmp, TLC) with apigenin. The fraction eluted with C_6H_6–EtOAc (9:1, 4:1) showed the presence of two components on TLC [C_6H_6–pyridine–HCO_2H (BPF), 36:9:5] which were further sepd by prep. TLC. One of these was comparable with kaempferol, mp 276–278°, R_f = 0.53 (BPF, 36:9:5) and the other was identical (mmp, MS, ^{1}H NMR, ^{13}C NMR) with authentic Et gallate. The C_6H_6–EtOAc (1:1) eluent gave a compound mp 315°, which was comparable (mp, TLC) with authentic quercetin.

Ethyl brevifolin carboxylate (1). Elution of the above column with EtOAc gave compound **1** as yellow needles (EtOAc–Me_2CO, mp 250°, brown colour under UV light and blue colour with $FeCl_3$, R_f = 0.5 [silica gel, toluene–HCO_2Et–HCO_2H (TEF), 5:4:1], IR ν_{max}^{KBr} cm^{-1}: 3400–3000, 1735, 1695, 1660, 1595, 1520, 1270, 1230, 1050. MS *m/z* (rel. int.): 320 (50, $[M]^+$, $C_{15}H_{12}O_8$), 274 (100). $[M-MeCH_2OH]^+$, 246 (30), 218 (40), 204 (20), 190 (16), 77 (20), 73 (20). ^{1}H NMR (DMSO $-d_6$): 1.18 (3H, *t*, J = 7.3Hz, Me), 4.08 (2H, *q*, J = 7.3 Hz, CH_2), 4.40 (1H, *dd*, J = 2 and 7.7 Hz, H-1), 7.30 (1H, *s*, arom, H), ^{13}C NMR (DMSO $-d_6$): 13.813 (Me), 37.037 (CH_2), 40.665 (CH), 60.473 (CH_2), 108.318 (arom CH), 113.114, 114.951, 138.387, 140.148, 143.518, 145.749, 149.711, 160.048, 171.887, 192.789 (arom C × 10).

Hydrolysis. Compound **1** (5 mg) was heated with 10% HCl. After 1 hr the yellow ppt. of brevifolin carboxylic acid appeared which was filtered and washed with H_2O and dried.

Methylation. To compound **1** (5 mg) in MeOH, CH_2N_2–Et_2O soln was added. After 6 hr solvents were removed and the reaction product crystallized from MeOH. The per Me ether of **1** melted at 136° and compared well with that of the tri Me ether of Et brevifolin carboxylate (lit mp 135°) [11]. The sample was not sufficient for spectral studies.

D-3-O-methyl *chiro*inositol (**2**). The MeOH ext. was dried and then treated with H_2O. The H_2O sol. fraction was transferred to a cellulose column and eluted with H_2O, H_2O–MeOH (1:1) and MeOH. Elution with H_2O–MeOH (1:1) followed by the crystallization from MeOH gave D-3-*O*-methyl *chiro*inositol as colourless cube-shaped crystals, mp 183°, R_f = 0.09 (TEF, 5:4:1), $[\alpha]_D^{H_2O}$ + 60, $C_7H_{14}O_6$ ($[M]^+$ *m/z* 194); IR ν_{max}^{KBr} cm^{-1}: 3300 (*br*, OH); MS *m/z* (rel int): 195 ($[M+1]^+$, 100), 194 ($[M]^+$, $C_7H_{14}O_6$), 177 (10), 159 (15), 127 (30), 109 (25), 86 (15); ^{1}H NMR (DMSO $-d_6$): δ 3.35 (*s*, 3H, OMe), 3.15–3.9 (6H, –CH proton), 4.26, 4.31, 4.43 (*q*), 4.63 (*q*, exchangeable D_2O), ^{13}C NMR (DMSO $-d_6$): δ 56.99 (*q*, CH_3), 68.002 (*d*, C-1), 70.48 (*d*, C-5), 71.903 (*d*, C-6), 72.131 (*d*, C-3), 73.27 (*d*, C-4), 80.180 (*d*, C-2).

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REFERENCES

1. Sastri, B. N. (ed.) (1984). *The Wealth of India* Vol. 1, p. 21. CSIR, New Delhi.
2. Kupchan, S., Morris, T. Mitsuo, S. R. M. and Steyn, P. S. (1971), *J. Org. Chem.* **36** 1972.
3. Inoue, T. Ishidata, Y. Fujeta, M. Kubo. M. Fukushima, M. and Nagai, M. (1978) *Yakugaku Zasshi*, **98**, 41.
4. Kubo, M. Nagai, M. and Inoue, T. (1983) *Chem Pharm Bull* **31**, 1917.
5. Nagai, M. Kubo, M. Fujita, M. Inoue T. and Matsuo, M. (1978) *Chem Pharm Bull* **26**, 2805.
6. Chunging, S. Ning, Z. Rensheng, Xu., Guoiang, S., Sheng yu and Shanhai, H. (1982) *Huaxue Xuebao* **40**, 1142.
7. Prakash, L. and Garg, G. (1981) *J. Indian Chem. Soc.* (A) LVII **90** (B) LVIII, 726.
8. Schmidt, O. T. and Eckert, R. (1956) *Z. Naturforsch* **11b**, 757.
9. Schimdt, O. T. and Bernauer, K. (1954) *J. L. Ann. Chem.* **588**, 211.
10. Schimdt O. T. Eckert, R. Guenther, E. and Fiesser, H. (1967) *J. L. Ann. Chem.* **706**, 204.
11. Schimdt, O. T. and Eckert, R. (1958) *J. L. Ann Chem.* **618**, 71.
12. Dorman, D. E., Angyal, S. J. and Roberts, J. D. (1970), *J. Am. Chem. Soc.* **92**, 1351.