Phytochemical Studies of *Haloxylon recurvum*

E. AHMED, A. MALIK*, N. RIAZ AND A. SHARIF
International Centre for Chemical Sciences
H.E.J. Research Institute of Chemistry,
University of Karachi, Karachi-75270, Pakistan

(Received 6th April, 2004, revised 11th May, 2004)

Summary: Seven compounds have been isolated for the first time from *Haloxylon recurvum* namely, octadecanoic acid (**1**), octacosonic acid (**2**), 1-triacontanol (**3**), triacontanoic acid (**4**), β-sitosterol (**5**), ursolic acid (**6**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**7**). Their structures have been elucidated by EIMS, HREIMS, FAB, HRFABMS, ^{1}H and ^{13}C NMR spectroscopic methods.

Introduction

Haloxylon recuruum Bunge ex Boiss (*H. Stockssii*) belongs to the family of Chenopodiaceae having 100 genera and 1200 species. Most of the members of this family are weedy and grow in waste places and unfertile areas of soil. In Pakistan this family is represented by 35 genera. Only five species of genus *Haloxylon* are found in Pakistan. *Haloxylon recuruum* is perennial shrub with glabrous leaves. It is widely distributed in Turkey, Syria, Iraq, Iran, Afghanistan, Kashmir, India and Central Asia. The plant is famous for its toxicity [1] and may in some cases kill insects [2]. The ash is used for internal ulcers [2]. No phytochemical work has been so for reported on this species. The diverse medicinal uses attributed to this species prompted us to carry out phytochemical studies. The methanolic extract of *H. recuruum* showed strong phytotoxicity. On further fractionation the major phytotoxicity was detected in chloroform soluble fraction. Phytochemical studies on chloroform soluble fraction have resulted in the isolation and structure elucidation of octadecanoic acid (**1**), octacosonic acid (**2**), 1-triacontanol (**3**), triacontanoic acid (**4**), β-sitosterol (**5**), ursolic acid (**6**) and β-sitosterol-3 *O*-β-D-glucopyranoside (**7**) respectively.

Results and Discussion

Column chromatography of the chloroform soluble fraction of *Haloxylon recurvum* resulted in the isolation and characterization of seven compounds five fatty acids including octadecanoic acid (**1**), octacosonic acid (**2**), 1-triacontanol (**3**), triacontanoic acid (**4**), β-sitosterol (**5**), ursolic acid (**6**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**7**). All of these have been reported for the first time from *Haloxylon recurvum*. The compounds **1-7** were respectively screened for phytotoxicity but none of these was found to be active. Therefore it can be concluded that the strong phytotoxicity of the chloroform soluble fraction is a combined effect of various constituents present therein.

Experimental

General

Column chromatography (CC): silica gel 70-230 mesh. Flash chromatography (FC): silica gel 230-400 mesh. TLC: pre-coated silica gel 60 F_{254} plates. UV: detection at 254 nm and by ceric sulphate reagent. Optical rotations: Jasco-DIP-360 digital polarimeter. UV and IR spectra: Hitachi-UV-3200 and Jasco-302-A spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR, spectra: Bruker spectrometers operating at 500, 400, 100 MHz; chemical shifts δ in ppm relative to $SiMe_4$ as internal standard and coupling constants J in Hz. EI-, FAB-, HR-EI-, HR-FAB-MS: JEOL JMS-HX-110 and JMS-DA-500 mass spectrometers, *m/z* (rel. int). The purity of the isolated compounds was check on pre-coated high performance thin layer chromatography (HPTLC) plates of E-Merck.

Plant Material

The whole plant of Haloxylon recurvum Bunge ex Boiss was collected from Cholistan desert near district Bahawalpur (Punjab), Pakistan in October, 2001 and identified by Dr. Muhammad Shafiq, Plant Taxonomist, Cholistan Institute of Desert Studies, Islamia University Bahawalpur, where a voucher specimen has been deposited.

*To whom all correspondence should be addressed.

Isolation

The whole plant (20 kg) was extracted thrice with MeOH (60L) at room temperature. The combined methanolic extract was evaporated under reduced pressure to obtain a thick gummy mass (750 g). It was suspended in water and successively extracted with *n*-hexane, chloroform, ethylacetate and *n*-butanol. The chloroform soluble fraction (35 g) was subjected to column chromatography over silica gel eluting with *n*-hexane, ethylacetate, ethylacetate-methanol and methanol in increasing order of polarity. The fractions obtained from *n*-hexane-ethylacetate (8.6:1.4) were combined and again chromatographed over silica gel using hexane-chloroform (7.7:2.3) to obtain octadecanoic acid (**1**). The fractions obtained from *n*-hexane-ethylacetate (8.0:2.0) gave three major spots on TLC, combined and rechromatographed over silica gel using hexane-chloroform in increasing order of polarity to obtain octacosonic acid (**2**), 1-triacontanol (**3**), and triacontanoic acid (**4**), repectively. The fractions obtained from *n*-hexane-ethylacetate (7.2:2.8) were combined and rechromatographed over silica gel using hexane-chloroform (6.0:4.0 & 7.0:3.0) as eluent to obtain β-sitosterol (**5**), and ursolic acid (**6**). The fractions obtained from chloroform-methanol (9.0:1.0) were combined and chromatographed over silica gel using chloroform-methanol (8.8:1.2) to obtain β-sitosterol 3-*O*-β-D-glucopyranoside (**7**).

Octadecanoic acid (1)

The fraction which eluted with *n*-hexane-ethylacetate (8.6:1.4) was chromatographed over silica gel and eluted with hexane-chloroform (7.7:2.3) to give compound **1**(22mg) which crystallized from chloroform, m.p 69-70°C; IR (KBr) ν_{max} (cm^{-1}); 3272-2620, 1714, 930; ^{1}H-NMR ($CDCl_3$, 400 MHz); δ: 2.23 (2H, t, *J* = 7.4 Hz, CH_2-2), 1.55 (2H, m, Hz, CH_2-3), 1.35-1.23 (28H, br s,14 X CH_2(C-4-C-17) and 0.81 (3H, t, *J* = 6.9 Hz, CH_3-18). EIHRMS; *m/z* 284.2715 $[M]^+$ (calcd for $C_{18}H_{36}O_2$, 284.2709). The physical and spectral data showed complete agreement with the literature [3].

Octacosonic acid (2)

The fraction which eluted with *n*-hexane-ethylacetate (8.0:2.0) was re chromatographed over flash silica gel and eluted with hexane-chloroform (7.5:2.5) to give compound **2**(18mg), crystallized from chloroform, m.p 90-91°C; IR (KBr) ν_{max} (cm^{-1}); 3284-2618, 1712, 924; ^{1}H-NMR ($CDCl_3$, 400 MHz); δ: 2.32 (2H, t, *J* = 7.5 Hz, CH_2-2), 1.60 (2H, m, CH_2-3), 1.27-1.18 (48H, br s,24 x CH_2(C-4-C-27) and 0.85 (3H, t, *J* = 6.6 Hz, CH_3-28); EIHRMS showed $[M]^+$ at *m/z* 424.4280 (calcd for $C_{28}H_{56}O_2$, 424.4276). The physical and spectral data coincided with literature values [4].

1-Triacontanol (3)

The fraction which eluted with *n*-hexane-ethylacetate (8.0:2.0) was subjected to flash chromatography using hexane-chloroform (7.2:2.8) as eluent to give compound **3**(18mg), m.p 86-87°C; IR (KBr) ν_{max} (cm^{-1}): 3440, 2925, 1054; ^{1}H-NMR ($CDCl_3$, 400 MHz); δ: 3.61 (2H, t, *J* = 6.5 Hz, CH_2-1), 1.54 (2H, m, CH_2-2), 1.29-1.23 (54H, br s 27 X, CH_2(C-3-C-29) and 0.86 (3H, t, *J* = 6.6 Hz, CH_3-30); EIHRMS showed $[M]^+$ at *m/z* 438.4801 (calcd for $C_{30}H_{62}O$, 438.4795). The physical and spectral data resembled the reported values [5].

Triacontanoic acid (4)

The fraction which eluted with *n*-hexane-ethylacetate (8.0:2.0) was subjected to flash chromatography (silica gel)using hexane-chloroform (8.8:2.2) to afford compound **4**(14mg), m.p 93-94°C; IR (KBr) ν_{max} (cm^{-1}): 3266-2636, 1708, 942; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ: 2.21 (2H, t, *J* = 7.5 Hz, CH_2-2), 1.52 (2H, m, CH_2-3), 1.23-1.17 (52H, br s 26 X CH_2(C-4-C-29) and 0.79 (3H, t, *J* = 6.5 Hz, CH_3-30). EIHRMS showed $[M]^+$ at *m/z* 452.4593 (calcd for $C_{30}H_{60}O_2$, 452.4585). The physical and spectral data showed complete agreement with the literature [4].

β-Sitosterol (5)

The fraction which eluted with *n*-hexane-ethylacetate (7.2:2.8) was chromatographed over flash silica gel and eluted with hexane-chloroform (7.0.3.0) to obtain compound **5**(24mg) which crystallized from acetone, m.p. 136-137 °C; IR (KBr) ν_{max} cm^{-1}: 3446, 3050, 1650; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ: 5.23 (1H, m, H-6), 3.32 (1H, m, H-3), 1.01 (3H, s, Me-19), 0.92 (3H, d, *J* = 6.2 Hz, Me-21), 0.84 (3H, t, *J* = 7.0 Hz, Me-29), 0.83 (3H, d, *J* = 6.5 Hz, Me-26), 0.81 (3H, d, *J* = 6.5 Hz, Me-27), 0.68 (3H, s, Me-18). EIHRMS: *m/z* 414.3861 (calcd for $C_{29}H_{50}O$, 414.3849). The physical and spectral data showed complete agreement with reported values [6-8].

Ursolic acid (6)

The fraction which eluted with *n*-hexane-ethylacetate (7.2.2.8) was chromatographed over

flash silica gel eluting with hexane-chloroform (6.0:4.0) to furnish compound **6** (16mg) as a colourless amorphous powder, IR (KBr) ν_{max} (cm^{-1}); 3510, 3050, 1697, 1635, 820; ^{1}H-NMR ($CDCl_3$, 400 MHz); δ: 5.11 (1H, m, H-12), 3.19 (1H, dd, $J_{ax,ax}$ = 10.0 Hz, $J_{ax,eq}$ = 4.5 Hz, H-3α), 1.20 (3H, s, Me-27), 1.07 (3H, s, Me-23), 0.94 (3H, s, Me-25), 0.91 (3H, d, J = 6.6 Hz, Me-30), 0.86 (3H, s, Me-26), 0.81 (3H, s, Me-24), 0.80 (3H, d, J = 6.8 Hz, Me-29);EIHRMS; *m/z* 456.3603 (calcd for $C_{30}H_{48}O_3$, 456.3595). The physical and spectral data showed complete resemblance with reported values [9,10].

β-Sitosterol 3-O-β-D-glucopyranoside (7):

The fraction which eluted with ethylacetate-methanol (9.0:1.0) was chromatographed over flash silica gel using chloroform-methanol (8.8:1.2) as eluent to afford compound **7**(12mg), as a colourless amorphous powder, $[\alpha]_D^{24}$ –45° (MeOH); IR (KBr) ν_{max} cm^{-1}: 3452, 3044, 1648. ^{1}H-NMR ($CDCl_3$, 400 MHz); δ: 5.13 (1H, m, H-6), 4.57 (1H, d, J = 7.3 Hz, H-1′), 3.85 (1H, m, H-3), 3.24-3.80 (m, Glc-H), 1.01 (3H, s, Me-19), 0.92 (3H, d, J = 6.1 Hz, Me-21), 0.83 (3H, t, J = 7.0 Hz, Me-29), 0.83 (3H, d, J = 6.4 Hz, Me-26), 0.81 (3H, d, J = 6.4 Hz, Me-27) and 0.68 (3H, s, Me-18); HRFABMS: *m/z* 577.4444 $[M+H]^+$ (calcd for $C_{35}H_{61}O_6$, 577.4438). The physical and spectral data showed complete agreement with reported values [6].

References

1. S. I. Ali, M. Qaiser, "*Flora of Pakistan*", No. 204. Department of Botany, University of Karachi, Karachi, pp. 189 (2001).
2. B. N. Sastri, "The Wealth of India", Council of Scientific and Industrial Research, New Dehli, Vol V, p. 5 (1959).
3. Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra, **1**,757B, 851A, 1169C (1992).
4. W. Cocker, S. J. Shaw, *J. Chem. Soc*, 5194 (1962).
5. M. A. Nogueira, J. S. D. Oliveira, S. Ferraz, *Phytochemistry*, **42**, 997 (1996).
6. A. A. Bernard, L. Eokes, *J. Org. Chem.*, **42**, 725 (1977).
7. I. Rubinstein, L. J. Goad, A. D. H. Clague, J. L. Mulheirw, *Phytochemistry*, **15**, 195 (1976).
8. H. L. Holland, P. R. P. Diakow, G. J. Taylor, *Can. J. Chem.*, **56**, 3121 (1978).
9. M. Hadaka, M. Ito, Y. Matsuda, H. Kohda, K. Yamasaki, J. Yamahora, *Phytochemistry*, **26**, 2023 (1987).
10. H. Budzikiewicz, J. M. Wilson, C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).
11. A. M. Iribarren, A. B. Pomilio, *J. Nat. Prod.*, **46**, 752 (1983).