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Chemical Constituents of the lichen *Stereocaulon tomentosum*

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ABSTRACT

A phytochemical investigation on the lichen *Stereocaulon tomentosum* has been conducted. Three compounds namely Atranorin (1), Glutinol (2) and Vulpinic acid (3) were isolated from the ethyl acetate extract of the plants. The structures of 1-3 were determined by NMR studies including from ^{1}H, ^{13}C, APT spectral data.

Keywords: lichen *Stereocaulon tomentosum*, Atranorin, Glutinol, Vulpinic acid, NMR.

INTRODUCTION

Lichens are the symbiotic organisms of fungi (mycobionts) and algae (photobionts) distributed worldwide [Huneck 1999]. Lichen Saccumulate large concentrations of products, particularly aromatic phenolic compounds, sometimes exceeding 20% of dry weight. The majority of these compounds originate from them ycobiont. The general resistance of lichens toinsects and microbial attack is attributed to the presence of lichen compounds (Lawrey 1986).The cortical presence of yellow-coloured compounds, such as pulvinic acid derivatives in lichens play a defensive role against the non-visually oriented small invertebrate herbivores (Rundel 1978).A lichen is not a single organism the way most other living things are, but rather it is a combination of two organisms which live together intimately. Most of the lichen is composed of fungal filaments, but living among the filaments are algal cells, usually from a green alga or a bacterium. In many cases the fungus and the alga which together make the lichen may each be found living in nature without its partner, but many other lichens include a fungus which cannot survive on its own -- it has become dependent on its algal partner for survival.

MATERIALS AND METHODS

TLC and preparative TLC were performed using per coated aluminum and glass plates with silica gel 60 F_{254}, where as column chromatography was carried out on silica gels 230-400 mesh. Spots and bands for compounds on TLC were detected using UV light. UV spectra were recorded on a UV- 1650PC spectrophotometer. X-ray structure determination was carried out by Bruker SMART APEX and the accompanying SHELXTL programming suite. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on JEOL and chemical shifts in ppm were referenced to internal acetone-d6 and $CDCl_3$, respectively. ^{1}H-^{1}H COSY and NOESY spectra were acquired using the standard JOEL software.

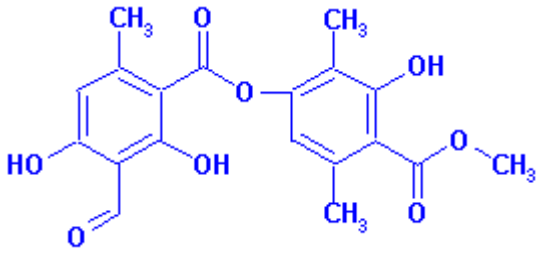
Plant material:

The *Stereocaulon tomentosum* were collected from a UKM forest. A voucher specimen had been deposited at the Herbarium of UKM.

Extraction and isolation: The air- dried powder (500 g) of **lichen** *Stereocaulon tomentosum* was extracted (Soxhlet) with ethyl acetate (2X, 10 hour each) and the

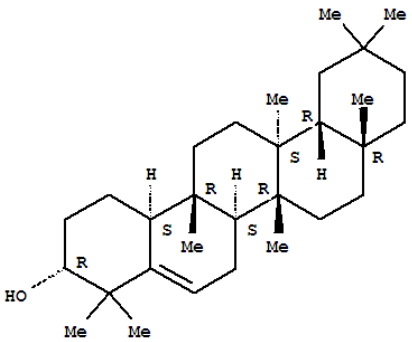
combined extracts evaporated to give a dark-green residue (15 g). this extract was subjected to column chromatography on silica gel with hexane containing increasing percentages of EtOAc as eluent and each collected fraction was 20 ml. fractions 3-6 contain Atranorin (1) (4.3 mg), R_f o.42 (hexane-EtOAc, 8:2). Fractions 5-13 (100 mg) were purified by radial chromatography with hexane- EtOAc (7:3) as eluent fractions 3 contain vulpinic acid (3) (3.0 mg), R_f 0.7(hexane-EtOAc). Fractions6-9 (30 mg) was purified further by preparative TLC with hexane-EtOAc (6:4) to afford Glutinol (2) (4.5 mg). Atranorin, Glutinol & vulpinic acid were identified by comparison with data from previous NMR and mass spectra.

Atranorin (**1**).Recrystallization from acetone gives light brown needles (3.5mg). Mp 194°C. UV ($CHCl_3$) λ_{max}nm (logε): 305 (0.29), 265 (1.06). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.57, 12.52, 12.05 (each 1H, Ar-OH), 10.37 (1H, -CHO), 6.53 (1H, Ar-H, H- 6'), 6.40 (1H, Ar-H, H-5), 4.00 (3H, s, -CO_2Me), 2.70 (3H, s, Ar-Me, C- 6), 2.55 (3H, s, Ar-Me, C- 5'), 2.10 (3H, s, Ar-Me, C-2'). Apt showed four methyl groups at δ 9.6, 24.3, 25.8 and 52.6 for Me- C2', Me- C5', Me- C6 and –CO_2Me. And also showed carbonyl ester at δ 169.3 (C-1) & 169.9 (C-4') and showed eldehyde group at δ 194.1 (C-3), (Hylands & Ingolfsdottir 1985).



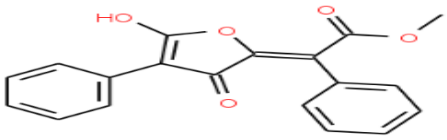
(1)

Glutinol (2): White powder amaterial, (13.8mg).m.p.212°C.(CHCl3) $\mathbb{m}_{max}$cm^1: 3436 (OH),1622.EIMS m/z (rel.int.): 426(55), 408(16), 274(100), 259(83), 205(42), 152(27) and 134(56); HREIMS m/z:426. 3818($C_{30}H_{50}O$, calcd.426.3812).^{1}H NMR ($CDC1_3$, 400 MHz): δ5.65 (1H, m, H-6), 3.72 (1H, m, H-3a), 0.85, 0.89, 1.00, 1.02, 1.05, 1.13, 1.14, 1.21 (each 3H, s); ^{13}CNMR ($CDC1_3$, 100 MHz): δ 140.68 (C-5), 121.96 (C-6), 76.68 (C-3), 49.83(C-10), 47.55(C-8), 43.21(C-18), 40.90 (C-4), 39.40 (C-14), 39.05(C-22), 37.95(C-13), 35.19(C-16 & C-19), 34.94(C-9), 34.72 (C-15), 34.60 (C-30), 33.24 (C-11), 32.46(C-28), 32.20 (C-21), 32.12 (C-29), 30.45 (C-12), 30.18(C-17), 29.04(C-23), 28.32 (C-20), 27.93 (C-2), 25.51(C-24), 23.74(C-7), 19.69(C-27), 18.47(C-26), 18.30 (1), 16.26 (25).(Gonzalezetal.,1987).



(2)

Vulpinic acid (**3**): yellow needles, mp (150-152 ^{o}C), UV (acetone) λ_{max} nm (log ε): 259 (0.05), 361 (3.01), 375(2.53); EIMS for $C_{19}H_{14}O_5$ m/z (rel. int.) 322(22.6%), 290(81.1%), 145(78.1%), 117(28.6%),89(100%), 63(20.3%). ^{1}H NMR(acetone, 400MHz) 3.92 (s, 3H, H-1), 7.34-7.49 (m,5H,aromatic), δ8.55 (s, 1H,H-13); ^{13}C NMR(acetone, 100MHz) δ55.1(C-1), 117.2(C-3), 128.5, 128.8, 129.3, 131 (m, ar. Carbons), 155.1(C-10), 161.7 (C-13), 164,9 (C-12), 166.5 (C-2), 172.8 (C-11).(Duncan 2003& Abo-Khatwa 1996)



(3)

RESULT AND DISCUSSION

Purification of the extract of lichen *Stereocaulon tomentosum* afforded compounds, namely atranorin (1), Glutinol (2) and vulpinic acid (3).

Compound 1 showed strong absorption in its UV spectrum at 305 (0.29) and 265 (1.06). The ^{1}H NMR displayed four methyl groups at δ 2.10, 2.55, 2.70 and 4.00. It also showed an aldehydic proton at δ 10.37. ^{13}C NMR spectrum displayed carbonyl ester groups at δ 169.3 and 169.9. It also showed a carbon aldehyde group at δ 194.1. Atranorin is a major component normally found in lichens (Quilhot W. *et al*., 1975; Culberson C.F. et. al., 1977; Faik A. et. al., 2008; Lumbsch H. T., 1995).

Compound 2; was isolated as white powders. The mass spectral data of the compound gave a molecular formula C_{30} $H_{50}O$, m/z 426. ^{1}H NMR (400 MHZ, $CDCL_3$) spectra showed the presence of eight methyl's appeared at δ 0.85, 0.89, 1.00, 1.02, 1.05, 1.13, 1.14, 1.21.The proton of H-3 appeared as a multiplet at δ 3.72. It also showed olefinicprotons at δ 5.65. ^{13}C NMR showed thirty carbons signal including eight methyles, ten methylenes, five methins and seven quaternary carbons. The alkenes carbons appeared at δ 140.68, 121.96.

Compound 3; was obtained as yellow needles, which determined by EIMS,^{1}H and ^{13}C NMR. The molecular formula of compound 3 was established by EIMS as $C_{19}H_{14}O_5$ which indicated 13 degree of instauration, (M^{+}, m/z 322), melting point 150-152 ^{o}C, and showed strong absorption in its UV spectrum. Suitable structure for X-ray analysis is in figure 2. The ^{1}H NMR displayed aromatic.

Group at δ3.92 (s, 3H).The ^{13}C NMR showed 19 carbon signals. The carbonyl carbons of vulpinic acid were located at δ166.5 and 172.6 for C-12 and C-2 respectively. Olefinic carbons were also observed at δ117.1 and 161.7 for C-3 and C-10 (Nakanishi 1998, Kanokmedhakul2003 and Clark 1999).

CONCLUSION

The isolation and identification of Atranorin (1), Glutinol (2) and vulpinicacid (3) from the lichen *Stereocaulon tomentosum* was the first ever to be done on this plant. The work was carried out by means of various physical (solvent extraction, column chromatography, radial chromatography, preparative TLC, malting points and R_f value) and spectral techniques.

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ARABIC SUMMARY

العناصر الكيميائية لاشته ستيريو كولون تومانتوس (lichen ***Stereocaulon tomentosum*****)**

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اظهرت الدراسة الطيفية لنبته ستيريو كولون تومانتوس (**lichen** *Stereocaulon tomentosum*) فصل ثلاثة مركبات وهي حيث تم عزلها باستخدام محلول خلات (Atranorin Glutinol, vulpinic acid) الايثيل للعينه النباتية. حيث تم التعرف على البنيه التركيبية للمركبات الثلاثة بواسطه الرنين المغناطيسي