ISSN : 0974 - 7427 Volume 7 Issue 3

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 7(3), 2013 [102-106]

New and rare acylated flavone glycosides from the aerial parts of chrozophora rottleri

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ABSTRACT

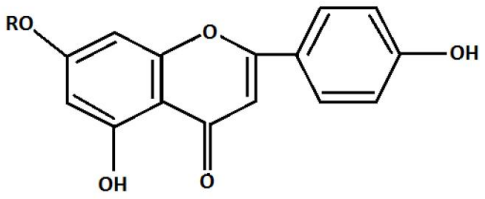
A new and a rare flavones glycosides, characterized as apigenin 7-O-β-D-(3"-E-p-coumaroyl) glucopyranoside and apigenin 7-O-β-D-(6"-E-p-coumaroyl) glucopyranoside respectively, in addition to known flavonoids apigenin, apigenin 7-O-methylether and apigenin 7-O-β-D glucopyranoside have been isolated from the aerial parts of chrozophora rottleri. Detailed ^{1}H NMR, ^{13}C NMR, as well as positive ESIMS data have been provided for the above compounds. © 2013 Trade Science Inc. - INDIA

KEYWORDS

Chrozophora rottleri;
Flavonoids;
Apigenin 7-O-β-D-(3"-E-p-coumaroyl) glucopyranoside.

INTRODUCTION

Chrozophora rottleri (klotzeh) is one of the eleven species of Euphorbiaceae growing in India and recorded as a medicinal plant possessing emetic, drastic and corrosive properties[1-4]. In the absence of any report on a systematic chemical examination of this plant and in continuation of our studies on the flavonoids of Indian medicinal plants[5-9], the aerial parts of *C.rottleri* were investigated for polyphenolics and the results leading the isolation of apigenin, apigenin 7-O-methylether, apigenin 7-O-β-D glucopyranoside, apigenin 7-O-β-D-(6"-E-p-coumaroyl) glucopyranoside and apigenin 7-O-β-D(3"-E-p-coumaroyl) glucopyranoside a new flavonoid are presented here.



Compound (1) R=H; Compound (2) R=OCH_3; Compound (3) R=β-D-glucopranosyl; Compound (4) R=6"-E-p-coumaroyl β-D-glucopyranosyl; Compound (5) R=3"-E-p-coumaroyl β-D-glucopyranosyl

RESULTS AND DISCUSSION

From the alcoholic extract of the air dried aerial parts five flavonoids were isolated.

Compound (1)

Compound (1) had fluorescence and UV maxima characteristic of an aglycone of flavones. ^{1}H-NMR spectrum showed evidence of a 5,7,4' –tri oxygenated flavone as well as the presence of protons at 2',

6',3',5',3 by a typical doublet pattern and a characteristic singlet for proton at C-3[10]. The ^{13}C-NMR spectrum of the compound further supported the above findings. Thus the structure of the flavone was established as 5,7,4' –trihydroxy flavone or apigenin.

Compound (2)

Compound (2) had fluorescence and UV maxima similar to that of compound (1), but the absence of characteristic bathochromic shift in band II of NaOAc compared to MeOH spectrum revealed that 7-OH is not free. On dementhylation with HI it gave compound (1). Thus the structure of compound (2) was established as apigenin 7-O-methylether[11] or genkwanin.

Compound (3)

Compound (3) had fluorescence and UV maxima characteristic of a flavone glycoside. On acid hydrolysis compound (3) yielded D-glucose and apigenin. The involvement of 7-OH in glycosylation was revealed by the absence of characteristic bathochromic shift in band II of NaOAc spectrum compared to MeOH spectrum. In addition the ^{1}H-NMR spectrum exhibited signals characteristic of apigenin glucoside. The ESIMS of the compound exhibited characteristic molecular ion peak at m/z 455 expected for the formula $C_{21}H_{20}O_{10}$[12]. Thus the structure of compound (3) was established as apigenin 7-O-β-D-glucopyranoside. It was earlier reported in *Euphorbia microphylla*.

Compound (4)

Compound (4) gave characteristic color reactions, R_f and spectral data of a flavones glycoside. On acid hydrolysis it yielded apigenin, D-glucose and p-coumaric acid in 1:1:1 ratio. The UV spectrum in NaOAc compared to MeOH spectrum had confirmed the glycosylation at C-7. The ^{1}H-NMR spectrum confirmed the 5,7,4' trioxygenated flavone structure of aglycone and also the involvement of 7-OH in glycosylation. The β-stereochemistry of the anomeric proton of glucosyl and the transstereochemistry of olefinic protons of p-coumaric acid were obtained from the characteristic chemical shifts as well as the coupling constants seen in ^{1}H-NMR data[14]. The site of esterification of glucose at C-6'' was confirmed by ^{13}C-NMR data. The appearance of C-6" at 64.1 ppm, a down fields shift of +3.4 comparing to C-6" of unsubstituted flavone glycoside at 60.7 ppm[12] and the appearance of C-5" (neighboring carbon) at 74.3 ppm, an unfiled shift -2.1 ppm in compound (4) comparing to C-5" of unsubstituted sugars at 76.4 ppm had confirmed the site of etherification at C-6" of glucose. The fragmentation pattern in ESIMS of the compound further supported the above findings, besides showing the molecular ion peak at m/z 570$(M+H)^+$ corresponding to the molecular formula of the compound (4) $C_{30}H_{10}O_{12}$ (required 578). From these data the structure of the rare flavonoid was established as apigenin 7-O-β-D-(6''-E-p-coumaroyl) glucopyranoside. Though there are earlier reports of this compound[15], it is reported for the first time from the species of *Euphorbiaceae.*

Compound (5)

Compound (5) the second acylated flavone glycoside was found to be a new natural product. Its characteristic color reactions, the products obtained on acid hydrolysis and the ^{1}H NMR data were found to be identical with that obtained for compound (4), but the R_f values on TLC (cellulose) developed with BAW (4:1:5 upper) showed slight difference (R_f, 88) from that of compound (4) (R_f 84). It suggests that it must be an isomer of Compound (4). This was further confirmed by the identical UV and ESIMS data of this compound with that of compound (4).

Further, the site of glycosylation at C-7, the β-linkage of anomeric proton of glucosyl moiety and the trans stereochemistry of olefinic protons of p-coumaroyl moiety were confirmed from the characteristic chemical shifts as well as from the coupling constant values obtained from ^{1}H-NMR data of this compound. The site of esterification at C-3'' of glucose was confirmed by the careful comparison of ^{1}H-NMR values of this compound with that of chrysoeriol 7-O-β-D- (3''-E-P-coumaroyl) glucopyranoside[16,17]. The H-3" signal usually appear at δ 3.1-3.5 as multiple in unsubstituted sugars appeared at δ 5.05 in compound (5) was in close agreement with values reported for H-3'' at δ 5.06 in chrysoerial 7-O-(3"-E-p-coumaroyl) β-D-glycoside. It was also observed that the values are different from 4'' –Z-p-coumaroyl glucoside reported by Chaudri and Thakur[12]. This fact was further confirmed by ^{13}C-NMR data. The appearance of C-3" signal at 80.7 ppm, a down field shift of +3.5 ppm comparing to the C-3"

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signal of unsubtituted flavone glycoside at 77.2 ppm and the appearance of C-2" and C-4"(neighboring carbons) at 71.0 ppm and 67.5 ppm, an unfiled shift of -2.2 ppm and -2.1 ppm respectively in compound (5) comparing to C-2'' and C-4'' signals of unsubstituted sugars at 73.2 ppm 69.6 ppm[12] had confirmed the site of etherification at C-3" of glucose in compound (5). Based on all these facts, the structure of compound (5) was established as apigenin 7-O- β-D-(3'' –E-p-coumaroyl) glucopyranoside, a new natural product.

EXPERIMENTAL

Plant material

Fresh aerial parts were collected from Reddiarpalayam, Pondicherry in April 2012 and authenticated by the Department of Botany, K. M. Centre for P.G. Studies, Pondicherry, where a voucher specimen (No.1/99) was deposited.

Extraction and Isolation

The air-dried and coarsely powdered aerial parts of the plant were extracted thrice with boiling 95% EtOH (3x5L) and concentrated in vacuo to 200 ml. The aqueous extract obtained on keeping in an ice-chest for 24 hours gave a dark brown solid. This solid was subjected to CC on SiO_2 (100-200 mesh) with CH_2Cl_2, $(CH_3)2CO$, MeOH and increasing proportions of their binary mixtures as eluents and 50 fractions each of 100 ml were collected and examined by PC/TLC. Compound (1) 60 mg from fractions 5 and 6, compound (2) 30 mg from fractions 8-10 and compound (3) 60 mg from fractions 11-13 were obtained. Fractions 22-48 showed the presence of two compounds on TLC (Benzene: py:AcOH,36:9:5). These fractions were combined together and CC over sephadex LH-20 using MeOH as eluent. 24 fractions 10 ml each were collected and examined by PC/TLC. Compound (4) 50mg from fractions 7-14 and compound (5) 20mg from fractions 16 and 17 were obtained.

(a) Apigenin (1)

Light yellow needles, mp 348-350æ%C (MeOH-Me_2CO); purple under UV changing to yellow with NH_3; UV max (MeOH):267,296sh,336; (+NaOMe) 275,324,392; (+NaOAc) 274,301,376; (+NaOAc/H_3BO_3) 268,302sh,338; (+$AlCl_3$) 276,301,348,384; (+$AlCl_3$/HCl) 276,299,340,381 nm; ^{1}H-NMR(200.13 MHz, DMSO-d_6): 13.39(1H,s,OH-5),10.98 (1H,s,OH-7),8.34 (2H, d,J 7.8 H_Z, H-2', H-6'), 7.34 (2H, J 7.9 H_Z, H-3', H-5'), 7.20(1H, s, H-3), 6.89 (1H d,J 2.4 Hz, H-8), 6.60 (1H,d,J 2.45 H_Z H-6); ^{13}C-NMR (50.32 MH_Z, DMSO-d_6): 181.8 (s, C-4),164.3(s,C-7), 163.8(s, C-4), 161.3 9 (s,C-5), 157.34(s, C-9), 128.5(d,C-2,C-6), 121.2(s, C-1), 116.0 (d, C-3, C-5), 103.7 (s, C-10), 102.9(s, C-3), 98.9 (s, C-6), 94.0(s, C-8); ESIMS(Positive):m\z (rel int .%) 271(M+H$^+$,100).

(b) Apigein 7-O-methylether (2)

Yellow needles, mp 325-327æ%C(EtOAc-petrol); purple under UV and yellow under UV/NH_3; UV max (MeOH) : 268,293.326 ;(+NaOMe) 268,296, 326 ; (+NaOAc) 260, 301, 370 ;(+NaOAc/H_3BO_3) 268, 293,326 ;(+$AlCl_3$) 276,301, 3458, 382; (+$AlCl_3$/HCl) 276, 299,340, 381 nm; ESIMS; m/z (rel int .%) 285(M+H$^+$,100).

(c) Apigein7-O-β-D-glucopyranoside (3)

Pale yellow needles mp 251-253æ%C (MeOH-Me_2CO): 268,333; (+NaOMe) 245sh, 269, 301 sh,386 ; (+NaOAc) 256sh,267, 355, 386 ;(+NaOAc/H_3BO_3) 267,340 ; (+$AlCl_3$) 276, 300, 348 ;(+$AlCl_3$/HCl) 277, 299, 341, 382nm; ^{1}H-NMR (400 MHz, DMO-d6),7.9 (2H J 8.7 H,H-2',H-6'), 6.9 (2H, J 8.9 Hz, H-3, H-5'), 6.8(1H, d, J, 2.5 Hz, H-8), 6.7 (1H, s, H-3) 6.4. (1H,d, J 2.4 Hz, H-6) of aglycone; 5.05 (d- J 7.25 Hz, H-1), 4.45 (d,J 11 Hz, H -6α), 4.15(d, J 11.5 Hz, H-6β) 3.85 (m,H-5), 3.50 (m, H-4), 3.45 (m, H-3 H-2) of glucose ; ESIMS : m/z (rel.int.%) 455 (M+Na$^+$,100).

(d) Apigenin 7-O-β–D-(6''- E-p-coumaroyl) glucopyranoside (4)

Pale yellow needles, mp 337-339æ%C (MeOH) ; purple under UV changing to yellow under UV/NH_3 ; gave Positive molisch test; UV max (MeOH) : 268, 317; (+NaOMe) 287, 314,380; (+NaOAc) 286, 317, 380; (+NaOAc/HBO_3) 268, 318, (+$AlCl_3$) 277, 284, 309, 326, 380; (+$AlCl_3$/HCl) 278, 284, 309, 326, 380; ^{1}H-NMR (400 MHz, DMSO-d) : 7.86 (d, J 8.9 Hz H-2,H-6), 6.79 (d,J 9.4 Hz, H-3, H-5), 6.78(d, J 2.44

Hz,H-1), 6.72 (s, H-3), 6.44 (d, J 2.13 Hz,H-6), 6.78 (d, J 2.44 Hz, H-8), 6.72(s, H-3), 6.44 (d, J 2,13 Hz H-6) of aglycone ; 5.15 (d, J 7.33 Hz, H-1), 4.45 (d, J 12 Hz, H-6), 4.15 (d, J 1 1 .9 Hz, H-6),3.82 (m,H-5), 3.45 (m,H-5), 3,35(m, H-3 H-2) of glucose ; 7.48 (d,J 8.84 Hz, H-β trans), 6.29 (d, J 1 5 .9 Hz, H-trans), 7.35 (d,J 8.6 Hz, H- 2, H- 6,), 6.66 (d, J 8.84 Hz, H-5) of p- coumaroyl ; ^{13}C-NMR (100 MHz DMSO-d) : 165.2(s, C-2) 94.9 (s,C-3),103.0 (s, C-3)", 182.1 (s, C=O), 161.6(s, C-5) 99.8 (s, C-6), 163.0 (s, C-7), 94.9 (s, C-8), 157.3 (s, C-9), 105.7 (s, C-10), 121.0 (s,C-1), 129.1 (d, C-2, C-6), 116.3 (d, C-3, C-5), 161.6 (s,C-4) of aglycone ;99.9 (s, C-1),73.4 (s,C=O), 113.8 (s,C-α), 1445.5 (s, C-β), 124.9 (s, C_1),130.6 (d, C-2, C-6), 117.3(d, C-3 C-5) 160.9(s, C-4) of p-coumaroyl ;ESIMS (positive): m/z (rel int .%) 601(M+Na^+,100), 579(M+N^+,25),433 (glucoside+H, 30), 271 (aglycone, 35), 155 (p-coumaric acid+H,45).

(e) Apigenin7-O-β–D-(3''-E-p-coumaroyl) glucopyranoside (5)

Pale yellow needles, mp 338-340æ%C (MeOH) ; purple under UV changing to yellow under UV/NH_3; gave Positive molisch test ; UV max (MeOH) : 269, 317 ;(+NaOMe) 267, 319, 368; (+NaOAc) 268, 317, 380; (+NaOAc/HBO_3) 268, 318, (+$AlCl_3$) 278, 299, 327, 381, (+$AlCl_3$/H_3BO_3) 278, 298,326, 379 ; ^{1}H-NMR (400 MHz, DMSO-d_6) : 7.90(d,J 8.85 Hz, H-2', H-6'), 6.84 (d,J 8.24 Hz, H-3', H-5'), 6.81(d, J 2.14 Hz,H-8) 6.79 (s, H-3), 6.42 (d,J 2 .14 Hz, H-6) of aglycone ; 5.17 (d, J 7.323 Hz, H-1), 5.05 d, J 7 .32 Hz, H-3) 4.45 (d,J 12.5 Hz, H-6α), 4.20 (d,J 1 2.1 Hz,H-6β), 3.85(m, H-5), 3.75 (m, H-4), 3.45 (m.H-2) of glucose ; 7.56 (d, J 15.87 Hz, Hβ –trans), 7.54 (d, J 8.8 Hz,H-2,H-6), 6.78 (d, J 8.55 Hz, H-3, H-5), 6,38 (d,J 16.17 Hz, H-trans) of p- coumaroyl ; ^{13}C –NMR (100 MHz, DMSO-d6): 165.1 (s, C-2), 103.1 (s,C-3), 182.2(s,C =O), 161.5 (s,C-5) 99.7(s, C-6), 163.1 (s, C -7), 94.9 (s, C-8), 157.4 (s, C-9), 105.6 (s,C-10), 121.0 (s, C-1'), 129.0(d, C-2', C-6'), 1 16.4 (d, C-3', C-5'), 161.4 (s, C-4) of aglycone; 99.9 (s,C-1), 71.0 (s, C-2_, 80.7 (s, C-3_, 67.5 (s, C-4), 76.4 (s,C-5), 60.8(s, C-6) of glucose ; 166.5 (s, C=O), 113.7 (s, C-α), 144.9 (s, C-β), 125.0 (s, C-1),130.4 (d, C-2, C-6), 177.2(d, C-3, C-5), 160,9 (s, C-4) of p-coumaroyl ; ESIMS (positive) : m/z (rel. int.%) 601 (M + Na^+, 100), 579 M + H^+, 25), 433 (glucoside+ H^+, 30), 271 (agly+ H^+,50), 155 (p-coumaricacid+ H^+, 35).

CONCLUSION

In this study, we reported the isolation of five flavonoids from Euphorbiaceae; five of them were isolated for the first time from this species. The results of the preliminary assay revealed that the crude extract and the flavonoids from *Chrozophora rottleri* could be used for cough and colds.

ACKNOWLEDGMENTS

The authors are very thankful to the Department of Botany, K. M. Centre for P.G. Studies, Puducherry for having authenticated the fresh aerial parts were collected from the Municipal area of Reddiarpalayam, Puducherry in April 2012.

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