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A New Phenyl Tetraglucoside and Phenolic Diglucoside from *Picrorhiza kurroa Royle ex Benth*

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Abstract

Context

Picrorhiza kurroa Royle ex Benth (Scrophulariacae) is a small perennial herb distributed in the alpine Himalayan tract and in some tropical parts of India. The production of two compounds Phenyl tetraglucoside and Phenolic diglucoside bioactive in *Picrorrhiza kurroa*. It may suggest that it is containing an interesting bioactive compound.

Aim

This research aimed to characterize the new phenyl tetraglucoside and phenolic diglucoside compounds in *Picrorrhiza species (*kurroa *sp.)* found on the Himalayan and some tropical parts of India.

Subjects and Methods

Isolation of bioactive compounds through total methanol extracts. It has been done with spectral studies using thin layer chromatography. The structures of both compounds were elucidated on the basis of UV, IR, Nuclear Magnetic Resonance (H^1 and C^{13} NMR) and Mass spectroscopic studies.

Results

Elucidation showed that compounds found as a new Phenyl tetraglucoside, namely, as 3-methoxy-4 hydroxyphenyl-n-butanyl-o-α-L-glucopyranosyl-(4a→1b)-α-L glucopyranosyl-(4b→1c)-α-L-glucopyranosyl-(4c→1d)-α-L-glucopyranosyl-4d-(3'-methoxy-4-hydroxyphenyl)-n-pent-7', 9'- dien-11'-oate, Phenolic diglucoside and vanillyl glucoside.

Conclusion

Results of this study suggest that a *Picrorhiza kurroa* species is a potential source of various active compounds.

Keywords: *Picrorhiza kurroa*; Schrophulariaceae; Phenyl tetraglucoside; Phenolic diglucoside; Vanillyl glucoside

Introduction

Kutki consists of the dried rhizomes and roots of *Picrorhiza kurroa Royle ex Benth*. (Family Scrophulariaceae); a perennial, more or less hairy herb common on the north-western Himalayas from Kashmir to Sikkim. It is an important medicinal plant, used traditionally for treatment of fever, malaria, asthma and jaundice caused by environmental pollution. It is also useful in gastrointestinal urinary disorders, leukoderma, snake bite, scorpion sting and inflammatory affections [1,2]. In this present paper, the isolation and structure elucidation of new glucoside from rhizomes of *Picrorhiza kurroa* were described.

Experimental

Material and methods

The rhizomes of kutki were procured from local market of Hisar in June 2010 and authenticated by Dr H.B. Singh, Head Raw Material Herbarium & Museum, Ref. NISCAIR/RHMD/ Consult-2010-11/11/1413/11. A voucher specimen has been retained in Department of Pharmaceutical Science, Guru Jambheshwar University of Science & Technology, Hisar. The plant material was air-dried at room temperature and then powdered [3,4]. The chemicals and reagents used were of Qualigens and SD Fine, Mumbai, LR grade. Melting points were determined in centigrade scale in one end open capillary and uncorrected. UV spectra were recorded in methanol on Elmer EZ-301 spectrophotometer and λ_{max} values are in nm. IR spectra were recorded on Shimadzu FTIR-8201 spectrophotometer using KBr pellets and ν_{max} values are in cm^{-1} ^{1}HNMR and ^{13}CNMR

were recorded on Bruker Anance 400 spectrometer using deuterated dimethylsuifoxide (DMSO-d_6), deuterated benzene (C_6D_6) and deuterated chloroform ($CDCl_3$) as solvents with trimethyl silane (TMS). Fast atomic bombardment mass spectra (FABMS) data were recorded on JEOL SX 102/DA-6000 mass spectrometer.

Preparation of *P. kurroa* extracts: The air-dried powder (3 kg) of kutki rhizomes was exhausted successively by petroleum ether (60-80°C), chloroform and methanol by hot extraction process and then aqueous extract was prepared by maceration process in distilled water for 18 h. The liquids extract so obtained were concentrated in vacuum at 40°C. The extracts were stored in refrigerator at 4°C until used for experiment. The column was eluted with petroleum ether, chloroform and methanol to get following compounds [5].

Results

Compound NPK-1

Elution of the column with chloroform: methanol (75:25) gave pale yellow crystals of compound NPK-1, recrystallised from chloroform:methanol (1:1), 242 mg (0.058%), R_f =0.64, m.p=114-115°C (Table 1).

IR ν_{max} (KBr): 3450, 3392, 3260, 2925, 2845, 1721, 1635, 1515, 1451, 1365, 1284, 1161, 1072 cm^{-1}.

Position	^{1}H NMR	^{13}C NMR
1a	5.05 d (6.4)	102.65
2a	4.42 m	74.89
3a	3.72 m	72.41
4a	3.81 m	73.85
5a	4.77 m	77.37
6a	3.25 d (9.6)	62.89
1b	5.01 d (6.4)	101.92
2b	4.36 m	74.71
3b	3.70 m	70.21
4b	3.80 m	73.37
5b	4.74 m	77.25
6b	3.28 d (8.0), 3.26 d (6.4)	61.34
1c	4.97 d (5.6)	97.79
2c	4.38 m	74.26
3c	3.57 m	69.85
4c	3.77 m	73.22
5c	4.66 m	76.32
6c	3.17 d (10.4), 3.06 d (10.4)	61.09
1d	4.94 d (6.4)	92.91
2d	4.26 m	74.15
3d	3.46 m	73.19
4d	3.75 m	79.68
5d	4.63 m	75.25
6d	3.20 d (11.2), 3.22 d (11.2)	60.55
1	-	144.63
2	7.45 d (2.0)	133.87
3	-	165.57

4	-	151.80
5	6.91 d (8.4)	128.94
6	7.52 dd (2.0, 8.4)	123.75
7	2.51 t (4.9)	41.72
8	1.22 m	37.35
9	1.05 m	35.10
10	3.33 t (9.5)	63.01
1'	-	141.05
2'	7.47 d (2.0)	130.51
3'	-	160.25
4'	-	147.47
5'	6.88 d (8.4)	128.33
6' 7'	7.43 dd (2.0, 8.4) 5.15 d (9.6)	119.97 117.82
8'	5.12 d (9.6)	115.76
9'	5.10 d (9.6)	115.20
10'	5.08 d (9.6)	112.59
11'	-	170.06
OMe	3.68 brs, 3.65 brs	58.45, 55.62

Table 1: ^{1}H-NMR and ^{13}C-NMR spectral data of compound NPK^{-1}.

Coupling constants in Hertz are provided in parenthesis.

ESI MS m/z (rel. int):1062 $[M]^+$ (C_{47} H_{66} O_{27}) (2.1), 739 (11.7), 203 (42.8).

Compound NPK-2

Elution of the column with chloroform:methanol (50:50) afforded pale yellow crystals of compound NPK-2 recrystallised from methanol, 254 mg (0.072%), Rf=0.58, m.p=108-109°C.

IR ν_{max} (KBr): 3455, 3389, 3270, 2925, 2845, 1703, 1633, 1516, 1450, 1376, 1283, 1160, 1074 cm^{-1}.

^{1}H-NMR (DMSO d_3):δ 17.43 (^{1}H, d, J= 2.4 Hz, H^{-2}), δ 9.81 (^{1}H, d, J = 4.5HZ, H^{-2}), 7.33 (^{1}H, dd, J = 2.4, 8.5 Hz, H^{-6}), 6.90 (^{1}H, d, J = 8.5, H^{-5}), 5.12 (^{1}H, d, J = 9.5Hz, H^{-7}), 5.10 (^{1}H, dd, 9.6, 8.8 Hz, H^{-8}), 5.07 (^{1}H, dd, J= 8.8 Hz, 11.2 Hz, H-9), 5.05 (1H, dd, J= 11.2, 4.5 Hz, H-10), 5.01 (^{1}H, d, J= 6.0 Hz, H$^{-1'}$) 4.92 (^{1}H, dd, J = 6.1Hz, H$^{-1''}$), 4.61 (^{1}H, m, H$^{-5'}$), 4.58 (^{1}H, m, H$^{-5''}$), 4.35 (^{1}H, m, H$^{-2'}$), 3.82 (^{1}H, m, H$^{-2''}$), 3.71 (^{1}H, m, H$^{-3'}$), 3.65 (^{1}H, m, H$^{-3''}$), 3.38 (^{1}H, m, H$^{-4'}$), 3.35 (^{1}H, m, H$^{-4''}$), 3.13 (^{2}H, dd, J = 8.0, 9.2 Hz, H$^{-6'}$), 3.02 (^{2}H, dd, J = 8.6, 7.3 Hz, H$^{-6''}$), 3.68 (3H, brs, OMe).

^{13}C-NMR (DMSO-d_6):δ 152.99 (C^{-1}), 148.54 (C^{-2}), 159.79 (C^{-3}), 154.37 (C^{-4}), 144.92 (C^{-5}), 143.85 (C^{-6}), 128.93 (C^{-7}), 128.40 (C^{-8}), 116.24 (C^{-9}), 115.58 (C^{-10}), 199.88 (C^{-11}), 102.63 (C$^{-1'}$) 81.82 (C$^{-2'}$), 73.35 (C$^{-3'}$), 70.15 (C$^{-4'}$), 77.36 (C$^{-5'}$), 62.90 (C$^{-6'}$), 101.93 (C$^{-1''}$), 75.26 (C$^{-2''}$), 72.43 (C$^{-3''}$), 69.16 (C$^{-4''}$), 76.68 (C$^{-5''}$), 61.02 (C$^{-''}$), 58.44 (OMe).

ESI MS m/z (rel. int):528 $[M]^+$ ($C_{24}H_{32}O_{13}$) (2.5).

Compound NPK-3

Elution of the column with methanol gave colourless crystals of NPK-3, recrystallized from acetone-methanol (1:1), 256 mg (0.068 % yield), R_f =0.76, m.p=95-96°C.

IR ν_{max} (KBr): 3390, 3260, 2930, 2853,1705, 1636, 1516, 1420, 1282, 1075 cm^{-1}

^{1}H-NMR (DMSO-d_6): δ 9.30 (^{1}H, brs, H^{-7}), 7.31 (^{1}H, d, J = 2.4Hz, H^{-2}), 6.48 (^{1}H, dd, J = 2.4, 8.5, H^{-6}), 6.02 (^{1}H, d, J = 8.5 Hz, H^{-5}), 4.96 (^{1}H, d, J = 6.2Hz, H$^{-1'}$), 4.67 (^{2}H, m, H$^{-5'}$), 4.19 (^{1}H, dd, 6.2, 6.9 Hz, H$^{-2'}$), 3.36 (^{1}H, m, H$^{-3'}$) 3.31 (^{1}H, m, H$^{-4'}$), 3.06 (^{2}H, dd, J = 9.5, 9.3Hz, H2$^{-6'}$), 3.20 (^{2}H, brs, OMe).

ESI MS m/z (rel. int): 314 $[M]^+$ ($C_{14}H_{18}O_8$) (2.6).

Discussion

Compound NPK-1, named Phenyl butanyl tetraglucosyl kurroate, was obtained as a pale yellow crystalline product from chloroform: methanol (3:1) eluant. It gave positive tests for glucosides and showed IR absorption bands for hydroxyl groups (3450, 3392, 3260 cm^{-1}), ester group (1721 cm^{-1}) and aromatic ring (1635, 1515, 1072 cm^{-1}). On the basis of mass and 13C-NMR spectra, the molecular ions peaks of NPK-1 was determined at m/z 1062 consistent to the molecular formula of a diphenyl substituted tetraglucoside, $C_{47}H_{66}O_{27}$ [6,7]. The

ions peaks arising at m/z 303 $[C_6H_3(OMe)\ (OH)\ (CH{=}CH)_2CO\text{-}]^+$ and 759 $[M\text{-}203]^+$ indicating the sugar unit, was esterified with pheny penanoate at one of the end. The ^{1}H-NMR spectrum of compound NPK-1 exhibited aromatic signals as one-proton doublets at δ 7.54 (J=4.0 Hz), 6.91 (J=8.4 Hz), 7.47 (J=2.0 Hz) and 6.88 (J=8.4 Hz) and one-proton double doublets at δ 7.52 (J=2.0, 8.0 Hz) and 7.43 (J=2.0, 8.4 Hz) assigned to H^{-2}, H^{-5}, H^{-2}, $H^{-5'}$, H^{-6} and $H^{-6'}$ protons respectively. Vinylic protons as one-proton doublets between δ 5.15-5.08, methoxy protons as three-proton broad singlets at δ 3.68 and 3.65, oxygenated methylene protons as a two-proton triplet at δ 3.33 (J=9.5 Hz) ascribed to H2-10 proton and other methylene proton triplet at δ 2.51 (J=4.9 Hz) and multiplets at δ 1.22 and 1.05. Four one-proton doublets at δ 5.05 (J=6.4 Hz), 5.01 (J=6.4 Hz), 4.97 (J=5.6 Hz) and 4.94 (J=6.4 Hz) were accounted to aromatic H^{-1}a, H^{-1}b, H^{-1}c and H^{-1}d protons, respectively. The other suger protons appeared between δ 4.77-3.06. The ^{13}C-NMR spectrum of compound NPK-1 displayed signals for ester carbon at δ 170.06 ($C^{-11'}$), aromatic and vinylic carbons between δ 165.57-112.59, methoxy carbons at δ 58.45 and 55.62, oxygenated methylene carbon at δ 63.01 (C^{-10}), methylene carbons at δ 41.75 (C^{-7}), 37.35 (C^{-8}) and 35.10 (C^{-9}), aromatic carbons at δ 102.65 (C^{-1a}), 101.92 (C^{-1b}), 97.79 (C^{-1c}) and 92.91 (C^{-1d}) and other sugar carbons from δ 79.68 to 60.55. The presence of C^{-4a}, C^{-4b}, C^{-4c} and C^{-4d} carbon signals at δ 73.85, 73.37, 74.26 and 79.68 respectively, in the deshielding region suggested attachment of sugar units in (1→4) linkages and location of the ester carbons at C-4d. On the basis of these evidences the structure of NPK-1 has been formulated as 3-methoxy-4 hydroxyphenyl-n-butanyl-o-α-L-glucopyranosyl-(4a→1b)-α-L glucopyranosyl-(4b→1c)-α-L-glucopyranosyl-(4c→1d)-α-L-glucopyranosyl-4d-(3'-methoxy-4-hydroxyphenyl)-n-pent-7', 9'- dien-11'-oate. It is a new phenyl tetraglucoside (Figure 1).

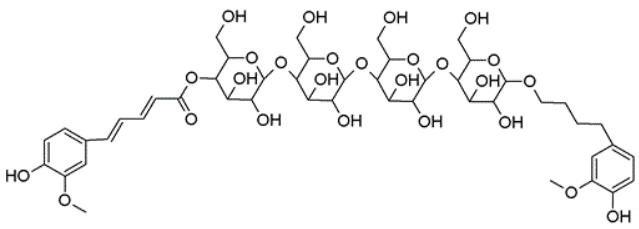

Figure 1: Phenyl butanyl tetraglucosyl kurroate.

Compound NPK-2, named Picraldehyde diglucoside, was obtained as a pale yellow crystalline product from chloroform:methanol (1:1) eluant. It gave positive tests for glycoside and showed IR absorption bands for hydroxyl groups (3455, 3389, 3270 cm^{-1}), aldehydic function (1703 cm^{-1}) and aromatic ring (1633, 1516, 1074 cm^{-1}). On the basis of mass and 13C-NMR spectra, the molecular ion peak of NK-2 was determined at m/z 528 corresponding to the molecular formula of a phenolic aldehydic diglucoside, $C_{24}H_{32}O_{13}$. The ^{1}H-NMR spectrum of NP-2 showed three one-proton doublets at δ 9.81, (J=4.5 Hz), 7.43 (J=2.4 Hz) and 6.90 (J=8.5 Hz) and a one-proton double doublet at δ 7.33 (J=2.4, 8.5 Hz) assigned to H^{-11} aldehydic and aromatic H^{-2}, H^{-5} and H^{-6} protons respectively. A one-proton doublet at δ 5.12 (J=9.5 Hz) and three double doublets at δ 5.10 (J=9.6, 8.8 Hz), 5.07 (J=8.8, 11.2 Hz) and 5.05 (J=11.2, 4.5 Hz) were attributed correspondingly to vinylic H^{-7}, H^{-8}, H^{-9} and H^{-10} protons. Two one-proton doublets at δ 5.10 (J=6.0 Hz) and 4.92 (J=6.1 Hz) were accounted to aromatic $H^{-1'}$ and $H^{-1''}$ protons, respectively. The other sugar protons appeared between δ 4.58-3.02. A three-proton broad singlet at δ 3.68 was due to methoxy protons. The ^{13}C-NMR spectrum of NP-2 exhibited signals for aldehydic carbon at δ 199.88 (C^{-11}), aromatic and vinylic carbons between 159.79-115.58, aromatic carbons at δ 102.63 ($C^{-1'}$) and 101.93 ($C^{-1''}$), other sugar carbons from δ 81.82 to 61.02 and methoxy carbon at δ 58.44. The presence of sugar $H^{-2'}$ proton in the deshielded region at δ 4.35 and carbon $C^{-2'}$ proton at δ 81.82 indicated (1→2) linkage of the sugar units. On the basis of these evidences, the NPK-2 has been formulated as 3-methoxy-4-hydroxyphenyl-n-pent-7,9-dien-11-al-4-α-L-glucopyranosyl (1→2)- α-L- glucopyranoside. This is a new phenolic diglucoside (Figure 2).

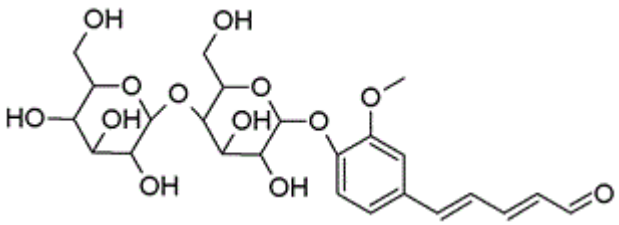

Figure 2: Phenolic diglucoside.

Compound NPK-3, named Vanillyl glucoside was obtained as a colourless crystalline product from pure methanol eluant. It gave positive tests for aldehyde and glucosides and showed IR absorption bands for hydroxyl groups (3390, 3260 cm^{-1}), aldehydic function (1705 cm^{-1}) and aromatic ring (1636, 1516 and 1075 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at m/z 314 corresponding to aromatic aldehydic glucoside $C_{14}H_{18}O_8$. The ^{1}H-NMR spectrum displayed a one proton broad singlet at δ 9.30 assined to aldehydic H^{-7} proton, two one-proton doublets at δ 7.31 (J=2.4Hz) and 6.02 (J=8.5Hz) and a one-proton double-doublet at δ 6.48 (J=2.4, 8.5Hz) ascribed to aromatic H-2, H-5 and H-6 respectively, a one-proton doublet at δ 4.96 (J=6.2Hz) attributed to α-oriented aromatic $H^{-1'}$ proton, other protons between δ 4.19 to 3.06 and methoxy carbon at δ 3.20. On the basis of above discussion, the structure of compound NP-3 has been elucidated as Vanillic-4 α-L-glucopyranoside (Figure 3).

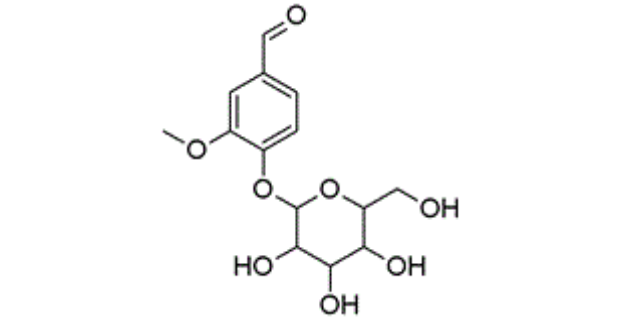

Figure 3: Vanillyl glucoside.

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Conflicts of Interest

There are no conflicts of interest.

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