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RESEARCH ARTICLE

IDENTIFICATION OF NOVEL GLYCOSIDIC COMPOUNDS OF *BUTEA MONOSPERMA* (BARK)

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ABSTRACT

Butea monosperma belongs to family leguminoaceae. It is locally called as Palas, Dhak and Khakhra. A lot of scientific literature is also available in which extract of its bark was used as astringent in diarrhoea. Here, we report three new compounds from EtOAc: MeOH and MeOH fraction of ethanol extract. 20-hydroxy noneicosan-7-oate, 3-O-β-D- glucopyranosyl-urs-12-ene-27, 28-dioic acid and 22.23-dihydro-4-stigmastene-3-β-D-glucopyranoside were the identified by spectral (IR, ^{1}HNMR spectra, mass spectrum) and chemical analysis. These are novel compounds and being reported first time by us

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INTRODUCTION

Butea monosperma (Lam) Kuntze commonly known as Flame of the Forest belongs to family leguminoaceae. It is locally called as Palas, Dhak and Khakhra. It is medium size tree commonly found throughtout India[1], except in the arid region. Bark, flowers, seeds, gum and leaves of *B. monosperma* are used in indigenous system of medicine for the treatment of various diseases. The flowers in the dried condition are known as Tesu , Kesu and Palas ke phool, have a bright orange colour but *B. monosperma* tree with pale yellow colour flowers has been reported for the first time from Bhalua forest in Gaya forest division[2], although they are much layer, closely resemble in appearance to the common gorse (Wex european) with, they are botanicaliy allied. *B. monosperma* is also called as medicinal dye bearing tree[3]. The bark of this plant are astringent, pungent, alternative, aphrodisiac, anthelmintic and used in ulcer and tumours. It is also used against house flies[4]. Decoction of bark is given in cold, cough, fever, haemorrhage, menstrual disorder, piles to cure "Vata and Kapha". Alcoholic extract of bark is given in bloody diarrhoea. Its dried flowers of this plant are called as tisso, tesoo, keeso, kesaree flower and Tesu ke Phool in Hindi and are used in making colour. In India, where they are used in the production of beautiful yellow and orange, red dyes, but the tints are not permanent[5]. Flowers and young fruits are used as vagetables by tribals and flower are boiled in water and cooked to obtain a dye[6]. Flowers are astringent, sweet, laxative, anthelmintic and tonic, aphrodisiac and diuretic[7]. Green leaves of this plant are good fodder for domestic animals and are used for making dinning plates and bowls. The complete mature leaves are also used for making Ghongda to protect from rains. Fresh twings are tied on horns of bullocks on occasion of 'Pola' festival[6] Leaf powder, about two spoonfuls mixed with a cup of water per day for a month is drunk mixed with a cup of water to cure diabetes[8].A hot poultice of the leaves is effectively used as antiphlogistic or dispersing boils, pimples, tumours haemorrhoids, ulcer, swelling etc. in case of retention of urine the region of the bladder is fermented with hot leaves. The leaves are also used in inflammation in eye diseases[9-10]. A euphane triterpenoid ester and a pterocapan were reported from the leaves of this plant[11].

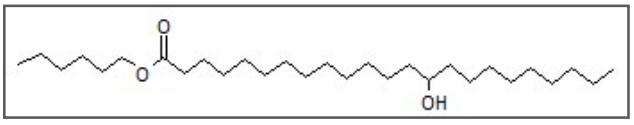
EXPERIMENTAL

General procedures: Melting points (mp) are uncorrected. 1H NMR was recorded on 300 MHz Varian XL spectrometer, ^{13}C NMR spectra were recorded on Varian XL 75 MHz spectrometer, IR spectra were recorded in KBr disk on Perkin Elmer-377 spectrometer, EIMS on Jeol-JMS D 300 mass spectrometer. All chemical shifts (δ) are given in ppm and Me4Si was used as internal standard. The carbon type (CH_3, CH_2, and CH) was determined by DEPT experiments. Chemicals are of analytical-reagent grade and column chromatography was carried out on silica gel and TLC on

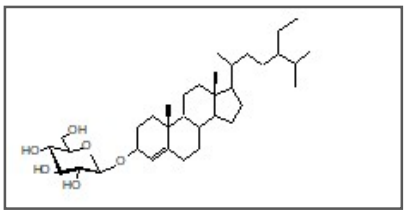
silica gel G (CDH/Glaxo laboratories). Spots were isualized by exposure to iodine vapor or by spraying with H_2SO_4-vanillin solution followed by heating at 105°C for 5 min.

Plant material: The bark of *B.monosperma* were collected from the nearby area of Ujjain city and identified by authorities of IEMPS, Vikram University, Ujjain. The bark was shade dried and ground to powder. Powered bark (12 kg) was exhaustively extracted in soxhlet extractor with benzene, benzene: acetone and ethanol.

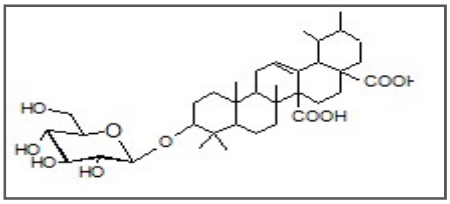
Extraction and isolation: The bark powder (12 kg) were shade dried, cleaned, coarsely powdered and extracted with n-hexane, benzene, ethyl acetate and ethanol in soxhlet-extractor for 72 h. Removal of solvent under reduced pressure afforded solid extracts. The yield of ethanol extract was quite good it was taken for the isolation of active compounds by repeated column chromatography. The three compounds are isolated from EtOAc: MeOH and MeOH fraction of ethanolic extract by repeated column chromatography on silica gel. The column was eluted by gradient elution in increasing order of polarity like hexane, benzene, ethyl acetate was eluted by gradient elution in increasing order of polarity like hexane, benzene, EtOAc and methanol. EtOAc: MeOH fraction was rechromatographed on silica on the basis of increasing order of polarity of eluents. The column was successively eluted with the hexane, benzene, EtOAc and methanol and their mixtures of increasing polarity. Fractions (a and b) (hexane: EtOAc v/v 6:4 and EtOAc:MeOH v/v 9:1) were purified and identified as 20-hydroxy noneicosan-7-oate and 22.23-dihydro-4-stigmastene-3-β-D-glucopyranoside. The compound was analyzed by IR, ^{1}H NMR, ^{13}CNMR and mass spectrometry with the literature data. MeOH fraction was rechromatographed on silica on the basis of increasing order of polarity of eluents. The column was successively eluted with the hexane, benzene, $CHCl_3$ and methanol and their mixtures of increasing polarity. Fractions (c) ($CHCl_3$:MeOH v/v 1:1) were purified and identified as 3-O-β-D- glucopyranosyl-urs-12-ene-27, 28-dioic acid. The compound was analyzed by IR, ^{1}H NMR, ^{13}CNMR and mass spectrometry.



20-hydroxy noneicosan-7-oate



22.23-dihydro-4-stigmastene-3-β-D-glucopyranoside



3-O-β-D- glucopyranosyl-urs-12-ene-27, 28-dioic acid

Compound 1: 20-hydroxy noneicosan-7-oate (1) EIMS m/z (% intensity) 454 (M+) 454(6.9), 437(7.0), 435(6.0), 425(5.0), 404(5.0), 394(6.0), 377(7.8), 337(8.0), 327(14.0), 316(34.0), 302(20.0), 301(81.0), 290(18.9), 289(88.0), 272(9.0), 256(14.5), 247(13.0), 239(28.0), 228(9.0), 222(9.0), 216(10.0), 202(28.0), 192(10.0), 188(8.9), 180(15.0), 174(28.0), 162(33.2), 148(100.0), 146(44.0), 144(30.0), 138(95.0), 131(22.0), 128(32.0). $C_{29}H_{58}O_2$ (30 mg, $CDCl_3$) m.p. 215°C, isolated from EtOAc: MeOH (1:1, v/v) fraction, TLC Benznene:ether (8:2 v/v) as solvent system, it showed single clear spot. IR (KBr) tmax: 3478, 2917, 2849, 2352, 1713, 1645, 1574, 1472, 1297 and 730-719 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): 0.88 (t, 6H, J = 7.5 Hz, $2CH_3$), 3.72 (s, 1H, -CH, J = 6.9 Hz), 4.05 (t, 2CH,-CH_2OCO, J=8.1 Hz), 2.32 (t, 2H, CH_2COO, J=8 Hz), 1.60 (s, 1H, -OH), 1.25 (s, 54H, -$27CH_2$).

Compound 2: 22.23-dihydro-4-stigmastene-3-β-D-glucopyranoside (2) EIMS m/z (% intensity) M^+ 576 (414 (aglycone) $C_{29}H_{50}O$) 414(9.7), 389(6.0), 293(8.0), 279(8.0), 234(10.0), 209(18.2), 193(24.0), 148(30.0), 138(33.2), 121(22.0), 97(56.0), 87(24.0), 83(62.0), 70(80.0), 62(100.0), 56(70.0), 45(100.0), $C_{35}H_{60}O_6$30 mg, MeOH & DMSO) m.p. 215°C, isolated from EtOAc: MeOH (1:1, v/v) fraction, TLC Benzene: MeOH (8:2 v/v) as solvent system, it showed single clear spot. IR (KBr) tmax: 3399, 2959, 2933, 2361, 1638, 1460, 1368,1384,1166,1023 and 800-472 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): 5.38 (s, -C=CH), 3.68 (m, 1H, -CH, J = 6.5, 9.5Hz), 2.55 (d, 1H, C-18, J=11.6Hz), 1.28 (s, 3H, CH_3, C-27), 1.01 (s, 6H, -$2CH_3$, C-24 and C-26), 0.96 (s, 3H, CH_3, C-25), 0.89 (s, 3H, -CH_3, C-18), 0.94 (d, 3H, -CH_3, C-30, J = 7.6 Hz),). ^{13}C NMR spectrum (δ): 140.5, 36.3, 42.0 (-C), 70.1, 121.3, 31.6, 49.8, 56.3, 55.9, 36.0, 45.3, 29.4 (-CH), 39.0, 31.6, 24.0, 33.5, 20.6, 42.0, 24.0, 28.8, 39.4, 27.0, 22.7 (-CH_2), 11.7, 18.6, 18.9, 19.7, 19.1, 11.7 (-CH_3) and the Values of Glucose of compound (2) 100.9, 73.5, 77.2, 70.1, 76.8 and 61.1 ppm.

Compound 3: 3-O-β-D- glucopyranosyl-urs-12-ene-27, 28-dioic acid (3) EIMS m/z (% intensity M^+ 646 (484 (aglycone) $C_{30}H_{44}O_5$) 484(9.7), 434(20.0), 417(18.2), 389(6.0), 293(8.0), 279(8.0), 234(10.0), 209(18.2), 193(24.0), 148(30.0), 138(33.2), 121(22.0), 109(30.0), 97(56.0), 87(24.0), 83(62.0), 70(80.0), 71(99.0), 62(100.0), 56(70.0), 45(100.0). $C_{36}H_{54}O_{10}$ (20 mg, MeOH) m.p. 245°C, isolated from $CHCl_3$: MeOH (1:1, v/v) fraction, TLC Benzene: MeOH (8:2 v/v) as solvent system, it showed single clear spot. IR (KBr) tmax: 3421, 2981, 2344, 1687, 1454, 1386, 1317, 1075, 770 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): 5.59 (s, -C=CH), 3.95 (m, 1H, -CH, J 6.5, 9.5Hz), 2.25 (d, 1H, C-18, J=11.6Hz), 1.28 (s, 3H, CH_3, C-27), 1.01 (s, 6H, -$2CH_3$, C-24 and C-26), 0.97 (s, 3H, CH_3, C-25), 0.89 (s, 3H, -CH_3, C-18), 0.99 (d, 3H, -CH_3, C-30, J = 7.6 Hz), 0.83 (d, 3H, -CH3, C-29, J = 7.6 Hz). ^{13}C NMR spectrum (δ): 38.3, 40.1, 37.8, 133.9, 56.9, 49.6, 179.0, 181.6 (-C), 78.3, 52.5, 48.2, 130.5, 55.5, 40.4, 33.0 (-CH), 39.9, 28.5, 19.1, 37.6, 23.9, 28.8, 25.7, 31.2, 37.6(-CH_2), 23.8, 17.1, 15.9, 18.1, 19.1, 21.5 (-CH_3) and the value of glucone of compound (3) 106.7, 75.7, 78.3, 71.7, 77.7, 62.8 ppm.

RESULT AND DISCUSSION

The natural compounds were identified mainly by their IR, ^{1}H NMR, ^{13}C NMR and Mass spectrometry analysis including a comparison with the literature data. The mass spectrum of 20-

hydroxy noneicosan-7-oate indicated the molecular ion peak at m/z 454 suggesting its molecular formula $C_{29}H_{58}O_2$. The mass fragmentation was characteristic of long chain hydrocarbon. The abundant fragments at m/z 171 and 295 were formed by McLafferty rearrangement and α-cleavage of the ester group respectively confirming the position of ester group in the molecule. The separation of most of the peaks by 14 and or 28 mass units and appearance of CnH2n+1 and CnH2n-1 ion series also confirming its long chain aliphatic nature[12-13]. IR spectrum showed a band at 3478 cm^{-1} for the presence of –OH group in the molecule. Bands at 2917, 2849, 1463 cm^{-1} were due to the –CH stretching and bending vibrations. The band at 1713 cm^{-1} was for the ester and band at 1030 $cm^{-1}$for primary symmetric C-O-C stretching. Other bands at 1645, 1538, 1370, 1297, 729-719cm^{-1} confirm aliphatic ester nature of the compound[14-15]. The ^{1}H NMR spectrum showed triplet at δ 0.88 for six protons of terminal methyl groups. The triplet at δ 4.05 (J = 6.9 Hz) was due to α-methylene protons of the ester ($COOCH_2$) group. A triplet at δ 2.32 for methylene (CH_2COO-) protons adjacent to ester carbonyl. Peak at δ 1.60 was assigned to –OH proton and methylene protons β-to ester and hydroxyl group the carbinolic proton resonated at δ 3.72 and rest of methylene protons were resonated at δ 1.25 as an intense singlet[12-13]. Thus on the basis of the above evidences the compound is identified as 20-hydroxy noneicosan-7-oate, it is a novel compound and being reported first time by us. The mass spectrum 22.23-dihydro-4-stigmastene-3-β-D-glucopyranoside indicated the molecular ion peak for the aglycone was observed at m/z 414 and the molecular formula was thus calculated to be $C_{29}H_{50}O$. The fragmentation was typically that of sitosterol molecule. The diagnostically important peaks were obtained at m/z 398, 396, 384, 307, 273, 255 and 213 (255-ring D-fission) [16-17]. These fragments suggested the aglycone of compound to be a C-29 sterol with one double bond, one hydroxyl group and a C-10 saturated side chain. The IR spectrum revealed the presence of hydroxyl group in the molecule, (as broad band in the region 3399 cm^{-1} and bands at 1023 and 1073 cm^{-1}). The bands at 2959, 2933, 2870 and 1460 cm^{-1} were due to –CH stretching and bending vibration. A band at 1638 cm^{-1} indicated unsaturation in the molecule. Band at 1378 cm^{-1} revealed the presence of isopropyl group in the molecule. Thus the IR spectrum gave evidences, that the molecule may be a steroidal or terpenoidal type[18-19]. The NMR spectrum indicated the molecule to be a steroidal glycoside. The complexity of the signals in the region δ 3.7 to 4.2 indicated the presence of one or more sugar moieties in the compound. One proton doublet at δ 5.38 was attributed to the double bond at Δ^4 position in the ring 'A' of the steroidal nucleus[20]. There was no unsaturation observed for the side chain. A multiplet centered at δ 3.6 was attributed to carbinolic proton at C-3 and the deshielding was characterized for the glucosylation of the steroidal nucleus at C-3 position. The singlet at δ 0.70 and at 0.94 was attributed to the angular methyls at C-18 and C-19 position. The rest of the four secondary methyls at position C-21, C-26, C-27 and C-29 appeared as doublet at δ 1.05, 0.85, 0.89, and 0.96 respectively. The presence of one sugar was observed, as only one anomeric proton appeared in the spectrum resonating at δ 4.94 (J=7.6Hz)[21]. The sugar attached at C-3 was characterized to be glucose by acid hydrolysis followed by paper chromatogrphy. The protons of sugar resonated as δ 4.92, 4.28, 4.26, 3.71, 3.67 and 4.94 (J=2.5, 11.9 Hz). The PMR spectra clearly indicated the aglycone of compound to be a sitosterol type with a glucose unit attached at C-3 position.

Table no.1. ^{13}C NMR Chemical Shift Values of 22.23-dihydro-4-stigmastene-3-β-D-glucopyranoside (Aglycone)

Carbon No.	C	CH	CH_2	CH_3
1			37.0	
2			31.6	
3		70.1		
4		121.3		
5	140.5			
6			24.0	
7			33.5	
8		31.6		
9		49.8		
10	36.3			
11			20.7	
12			42.0	
13	42.0			
14		56.3		
15			24.0	
16			28.8	
17		55.9		
18				11.7
19				18.6
20		36.0		
21				18.9
22			39.4	
23			27.0	
24		45.3		
25		29.4		
26				19.7
27				19.1
28			22.7	
29				11.7

Table 2. ^{13}C NMR Chemical Shift Values of compound 3 (aglycone)

Carbon No.	C	CH	CH_2	CH_3
1.			39.9	
2.			28.5	
3.		78.3		
4.	38.3			
5.		52.5		
6.			19.1	
7.			37.6	
8.	40.1			
9.		48.2		
10.	37.8			
11.			23.9	
12.		130.5		
13.	133.9			
14.	56.9			
15.			28.8	
16.			25.7	
17.	49.6			
18.		55.5		
19.		40.4		
20.		33.0		
21.			31.2	
22.			37.6	
23.				23.8
24.				17.1
25.				15.9
26.				18.1
27.	179.0			
28.	181.6			
29.				19.1
30.				21.5

^{13}C NMR spectrum (75 MHz, MeOH, ppm): The ^{13}C NMR spectrum revealed the aglycone to be a C-29 carbon skeleton with the presence of one double bond at 121.6 and 140.9 ppm. The deshielding observed for the C-3 carbon (77.1 ppm) was justified for the place of glycosilation at this position[12]. The

chemical shift values of C-2 (31.5 ppm) C-3 (76.7 ppm) and C-4 (29.4 ppm) revealed the presence of a β-oriented glucosyl moiety at C-3 position, by comparison with the same signals of aglycone[13]. The ^{13}C NMR values for the complete assignment of the molecule are listed in the table no.1, The compound 2 was confirmed to be a glucosylated β-lawseritol. And the Chemical Shift Values of Glucose of compound C-1' (100.9), C-2' (73.5), C-3' (77.2), C-4' 70.1, C-5' (76.8) and C-6' (61.1) ppm. Based on the above spectral evidences the compound was characterized as 22.23-dihydro-4-stigmastene-3-β-D-glucopyranoside and being reported for the first time by us.

The mass spectrum of 3-O-β-D- glucopyranosyl-urs-12-ene-27, 28-dioic acid indicated the mass spectrum showed molecular ion peak for the aglycone at m/z 448 and the molecular formula was found to be $C_{30}H_{44}O_5$. The formation of ions at m/z 208 and 234 supported the presence of 12-13 double bonds in the molecule. The diagnostically important peaks were obtained at m/z 436(M^+-3Me+H_2O), 417(436-H_2O), 392(M^+-2HCOOH), 389(436-HCOOH), 387(M^+-ring D fission), 371(417-HCOOH), 209(484-ring C fission), 192(M+-ring C fission) and 139(M+-ring B fission). The fragmentation suggested the aglycone of compound to be a triterpene with one double bond, two carboxylic acid groups and one hydroxyl group. The IR spectrum showed absorption bands at 3421 and 1657 cm^{-1} for the presence of hydroxyl –COOH group. The absorption at 1600 cm^{-1} indicated the unsaturation in the molecule. Bands in the region 2928, 2867, 1408 cm^{-1} were due to –CH stretching and bending vibration[22]. The high-resolution NMR spectra clearly revealed the presence of one sugar and ursane type triterpene. A one proton singlet at δ 5.59 was characteristic of the olifinic proton at C-12 in pentacyclic triterpene of the α and β amyrin series[23]. A multiplet centered at δ 3.95 was due to C-3 proton and the deshielded position of C-3 indicated the position of the glycosylation of the triterpenoid nucleus. The presence of a doublet at δ 2.25 for C-18 methine proton and resonance of methyl proton as ursane rather than oleanane revealed the molecule to be of ursane type and not oleanane type triterpene. The ursane are the positional isomer of the oleananes in which 29-methyl function is attached at C-19. The methyls at carbon number C-23, C-24, C-25 and C-26 appeared as singlet at δ 1.28, 1.01, 0.89 and 0.97 and C-29, C-30 appeared as doublet at δ-0.99 and 0.83 gave clear indication of the compound to be ursane type rather than to the oleanane type. The values for ^1H NMR discussed above were in consistent to the ursane molecule known as quinonic acid previously investigated from various plant sources and reported in literature[24]. The proton of the sugar resonated at δ 4.86 (1H, d, J=3.0 Hz), α-linked anomeric proton, δ 3.85 (1H, d, J=6 Hz), δ 4.31 (1H, d, J=7.5 Hz), δ 3.66 (1H, m, J=14.25 Hz), δ 4.28 (1H, d, J=7.5 Hz), 3.33 dd, (2H, J=14.8 Hz), 3.31 dd, (J=2.1, 2.4 Hz) and 3.31 dd, (J=2.1, 2.4 Hz). The CMR spectrum also favored the β-orientation of the C-3 hydroxyl group as the singal of β-carbinol carban appeared at 78.3 ppm. The position of double bond at C-12-C-13 was confirmed by the resonances at 138 and 133 ppm. The presence of –COOH signal appeared at 182 and 179 ppm was assigned to C-28 and C-27 position respectively. The six methyls appeared at their respective positions resonated at 33.3, 17.4, 17.1, 18.2, 19.3 and 21.4 ppm. The peaks at 106, 75.7, 78.3, 71.7, 78.3 and 62.8 ppm suggested glucose sugar attached to C-3 position[25]. The peak at 106 ppm assigned to anomeric carbon of β-D-glucoside[17].

The CMR clearly indicated the molecule to be an ursane type triterpene with sugar molecule and based on the above spectral evidences the compound 3 was characterized, as 3-O-β-D-glucopyranosyl-urs-12-ene-27, 28-dioic acid being reported earlier from this plant this plant. ^{13}C NMR Chemical Shift Values of Glucose of 3-O-β-D- glucopyranosyl-urs-12-ene-27, 28-dioic is C-1' 106.7, C-2' 75.7, C-3' 78.3, C-4' 71.7, C-5' 77.7 and C-6' 62.8 ppm. Based on the above spectral evidences the compound 3was characterized as 3-O-β-D-glucopyranosyl-urs-12-ene-27, 28-dioic and being reported for the first time by us.

CONCLUSION

From the survey of the literature to the best of our knowledge all the three compounds were novel and being reported first time by us from Butea monosperma (bark) and further examination of the constituents of this plant is currently in progress.

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