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Oxanthrone esters from the roots of *Cassia kleinii*

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Abstract

From the roots of *Cassia kleinii* two new oxanthrone esters, kleinioxanthrone-3 and kleinioxanthrone-4 have been isolated. Their structures were established as 1,8-dihydroxy-3-methyl-9(10H)-anthracenone-10-oxyhexadecanoate 3 and 2,6,7-trihydroxy-1,8-dimethoxy-3-methyl-9(10H)-anthracenone-10-oxydecanoate 4 respectively based on degradative and spectroscopic evidence.

Keywords: *Cassia kleinii*; Leguminosae; Kleinioxanthrone-3; Kleinioxanthrone-4

1. Introduction

Cassia kleinii W&A (Leguminosae family) has long been used in traditional medicine for its antihepatotoxic activity. We previously reported the isolation and structure elucidation of two new oxanthrone esters kleinioxanthrone-1 (**1**) and kleinioxanthrone-2 (**2**) from the aerial parts of *C. kleinii* (Anu and Rao, 2001). In a continuing study, the roots of *C. kleinii* were investigated to determine the distribution of compounds present in the plant. Two more new oxanthrone esters were isolated and identified from the roots of the plant. They are named as kleinioxanthrone-3 (**3**) and kleinioxanthrone-4 (**4**) respectively. Kleinioxanthrone-1 has also been isolated from the roots along with two known triterpenes, betulinic acid and dihydrobetulinic acid. Hydroxy anthraquinone derivatives are at present gaining importance as potent hepatoprotective drugs.

2. Results and discussion

Compound **3**, kleinioxanthrone-3, was isolated as yellow needles from hexane. mp 177–178 °C. The UV spectrum displayed bands at 435 and 295 nm. The IR spectrum showed absorption bands at 3500 cm^{-1} (–OH), 1620 cm^{-1} (chelated –C=O) and 1749 cm^{-1} (ester –C=O).

The 300 MHz ^1H NMR spectrum ($CDCl_3$) revealed two broad one proton singlets at δ 12.1 and δ 12.0 which are characteristic of two chelated hydroxyl groups. It showed an ABC pattern of multiplets at δ 7.8 (*J* 9 Hz), δ 7.7 (*J* 9 Hz), δ 7.3 (*J* 9 Hz) assigned to H-7, H-6 and H-5 of (**3**). Two broad singlets seen at δ 7.6 and δ 7.1 in the aromatic region were assigned to H-2 and H-4. The one proton multiplet at δ 3.9 was assigned to the oxymethine proton C-10 of an oxanthrone. The three proton singlet at δ 2.47 was characteristic of an aromatic methyl group. The triplet at δ 2.35 accounted for an –OCO$\underline{CH_2}$ group. The broad singlet at δ 1.25 which integrated to 26 protons was characteristic of methylene groups belonging to an aliphatic straight chain. The triplet at δ 0.85 was typical of a terminal –CH_3 group. This data except for the integration of the peak at δ 1.25 was exactly identical to that of kleinioxanthrone-2 (**2**) isolated from the aerial parts of *C. kleinii*. Both have the same chrysophanol-9-anthrone nucleus and differ only in the length of the aliphatic ester side chain at C-10.

3 was identified by comparing its spectral and physical data with that of **2**. The R_f of **2** is 0.47 and that of **3** is 0.5. (Solvent system hexane: EtOAc 98:2). The melting points of the two differ by 10 °C. The EIMS of **3** gave the molecular weight of the corresponding anthraquinone alone (M^+ 254) due to cleavage of the side chain to form the oxonium ion M^+ 255 (Monache et al.,

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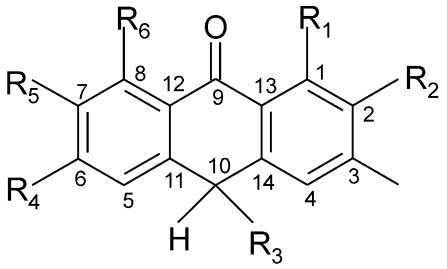
1987) and hence the anthraquinone. The side chain ester was deduced from the FABMS as hexadecanoic acid ester. The structure was confirmed by alkaline hydrolysis which yielded hexadecanoic acid. This was methylated to give its methyl ester which was compared by co-TLC and capillary GC with an authentic sample of methylhexadecanoate. Thus **3** was identified as 1,8-dihydroxy-3-methyl-9(10H)-anthracenone-10-oxyhexadecanoate.

Compound **4** crystallized from ethyl acetate as orange needles mp 196–198 °C. The molecular formula $C_{27}H_{34}O_8$ was deduced from the positive ion FAB MS. The IR spectrum showed absorption bands at 3400 cm^{-1} (OH), 1664 cm^{-1} (unchelated –C=O) (Thomson, 1987) and 1735 cm^{-1} (ester –C=O)

The 300 MHz ^1H NMR spectrum ($CDCl_3$) showed one proton singlet peaks at δ 7.77 and δ 7.13. Peaks at δ 4.13 and δ 3.99 which are three proton singlets are characteristic of $-OCH_3$ groups attached to an aromatic ring. The one proton broad singlet at δ 3.4 was assigned to an oxymethine at C-10 of an oxanthrone system. A three proton singlet peak at δ 2.35 is characteristic of a methyl group attached to an aromatic ring. The triplet at δ 2.1 accounts for a $OCOC\underline{H}_2$ group. The broad singlet at δ 1.25 is characteristic of methylene groups belonging to an aliphatic straight chain and integrated for 16 protons. The triplet at δ 0.82 was typical of a terminal $-CH_3$ group of an alkyl chain. The absence of peaks at δ 12.1 in the ^1H NMR spectra which is characteristic of hydroxyl groups at positions 1 and 8 proves that the carbonyl group is unchelated. By biogenetic consideration and comparison of structures with 1,6,8-trimethoxy-3-propanoylanthraquinone (Minocha et al., 1981) the two methoxyl groups are assigned at C-1 and C-8. The closeness in the chemical shifts of the methoxyl groups show that they are in similar environments. The two unsubstituted aromatic positions should be on different rings because they do not show any *ortho* or *meta* coupling. The methyl group is fixed at position 3 biogenetically and in comparison with other structures of oxanthrone esters obtained from the aerial parts of the plant. Two hydroxyls have to be adjacent to each other as is shown by the loss of a water molecule from the mass spectrum. The hydroxyl groups are therefore assigned to positions 2, 6 and 7. This left positions 4 and 5 as the unsubstituted positions of the aromatic ring. Thus the singlet at δ 7.13 is assigned to H-4 and that at δ 7.77 to H-5.

The EI MS of **4** gave the molecular weight of the corresponding anthraquinone alone (M^+ 330) which is formed via. the oxonium ion by the cleavage of the side chain. The FAB mass gave the molecular weight of the whole molecule $(M+H)^+$ 487. The size of the side chain can be deduced from this. Final confirmation of structure was obtained by hydrolysis of **4** with alcoholic KOH to give the aliphatic acid corresponding to the aliphatic ester side chain. This was identified as decanoic acid by co-TLC with that obtained from the hydrolysis of kleinioxanthrone-1. **4** is identified as 2,6,7-trihydroxy-1,8-dimethoxy-3-methyl-9(10H)-anthracenone-10-oxydecanoate a new oxanthrone ester named as kleinioxanthrone-4. The anthraquinone itself is a new compound.



1 $R_1=R_6=$ OH $R_2=R_5=$ H $R_4=$ OCH_3

$R_3 = OCO(CH_2)_8CH_3$

2 $R_1=R_6=$ OH $R_2=R_4=R_5=$ H

$R_3 = OCO(CH_2)_{12}CH_3$

3 $R_1=R_6=$ OH $R_2=R_4=R_5=$ H

$R_3 = OCO(CH_2)_{14}CH_3$

4 $R_1=R_6=$ OCH_3 $R_2=R_4=R_5=$ OH

$R_3 = OCO(CH_2)_8CH_3$

3. Experimental

3.1. Plant material

C. kleinii W&A (Hooker, 1978) was collected from Neyyatinkara, Thiruvananthapuram and identified by Dr. N. Sasidharan, Scientist, Kerala Forest Research Institute (KFRI), Peechi, Kerala and was deposited at the Herbarium of the KFRI and the voucher number is 7564.

3.2. Extraction and isolation

Dried and finely powdered roots of the plant were extracted (Soxhlet) with petrol (60–80 °C) and subsequently with chloroform for 48 h. The petrol extract

and the chloroform extract were evaporated and the residues were chromatographed separately over silica gel (60–120 mesh) using hexane and hexane:EtOAc (98:2, 93:7, 95:5, 90:10, 80:20) as eluents. **1** crystallized from the hexane:EtOAc (95:5) fraction of the hexane extract. **2** crystallized from the 93:7 fractions of the chloroform extract after repeated column chromatography.

3.3. Kleinioxanthrone-3 (*3*)

$[\alpha]_D^{25}$ −8° ($CHCl_3$, *c*, 1.20) UV: λ_{max} 435, 295 nm IR KBr ν_{max} cm^{-1} 3500 (OH), 1620 (chelated CO), 1749 (ester CO) FAB MS $(M+1)^+$ 495, M^+ 494 EI MS 254 (69), 168 (21), 150 (100), 128 (12), 70 (51), 56 (93), 42 (93) ^{13}C NMR (75 MHz $CDCl_3$) δ 162.3 (C-1), 115.7 (C-2), 149.1 (C-3), 124.1 (C-4), 124.2 (C-5), 136.8 (C-6), 119.7 (C-7), 162.2 (C-8), 192.5 (C-9), 68.2 (C-10), 133.3 (C-11), 123.1 (C-12), 121.2 (C-13), 133.5 (C-14), 22.5 (-CH_3), 181.7 (C-1′), 31.8 (C-2′), 22.2–30.0 (C-3′-15′), 13.4 (C-16′).

3.4. Kleinioxanthrone-4 (*4*)

$[\alpha]_D^{25}$ −21.5° ($CHCl_3$, *c*, 1.01) IR KBr ν_{max} cm^{-1} 3400 (OH), 1664 (unchelated CO), 1735 (ester CO)FAB MS $(M+1)^+$ 487, M^+ 486 EI MS 330 (100), 312 (55), 297 (22), 287 (18), 269 (12), 241 (22), 216 (22), 105 (15), 91 (90).

3.5. Hydrolysis of kleinioxanthrone-3 (*3*) and kleinioxanthrone-4 (*4*)

Kleinioxanthrone-3 (6 mg) and kleinioxanthrone-4 (2 mg) were taken separately in 2.5% alcoholic KOH (5 ml) refluxed for 1 h until an aliquot showed the disappearance of the original compound on TLC. It was then cooled and treated with water. The aqueous layer was neutralized with dil. HCl and extracted with ether and dried with Na_2SO_4. Hexadecanoic and decanoic acids were obtained from **3** and **4** respectively. Decanoic acid was identified by co-TLC with an authentic sample obtained from the hydrolysis of kleinioxanthrone-1.

3.6. Methylation of hexadecanoic acid

Hexadecanoic acid (2.3 mg) from the above hydrolysis was dissolved in methanol and 0.5 ml, 2% H_2SO_4 in methanol was added. The reaction mixture was refluxed for 30 min. It was then cooled, treated with water, extracted with hexane and washed with saturated sodium bicarbonate and water. The methyl ester obtained was identified by co-TLC and capillary GC with an authentic sample of methylhexadecanoate.

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