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ANTHRACENE DERIVATIVES OF *RHAMNUS PRINOIDES*

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ABSTRACT. The fruits of *Rhamnus prinoides* (Rhamnaceae) yielded the known anthracene derivatives physcion (**1**) emodin (**2**), emodinanthrone (**3**) and emodin bianthrone (**4**) in addition to the new emodinanthronediacetylrhamnoside derivative, prinoidin (**5**).

INTRODUCTION

Rhamnus prinoides (Amharic name: *Gesho*) is a cultivated indigenous shrub which is also known to occur as far west as Cameroon and as far south as S. Africa. The leaves and stems of the plant are essential ingredients in the domestic preparation of the fermented Ethiopian beverages *Tella* and *Tedj* respectively. However the role of this plant in the fermentation process is not clearly established (1). The fruits of *R. prinoides* are sold in Ethiopian medicinal plant markets for the treatment of fungal and ring worm infections (2). The only study reported in the literature on this plant is that of Salgues in 1962 which deals with the presence of inorganic cations, organic acids and the flavonoid derivative rhamnetin rhamnoside. He also found that the leaf extracts showed some toxic effects to rabbits (3).

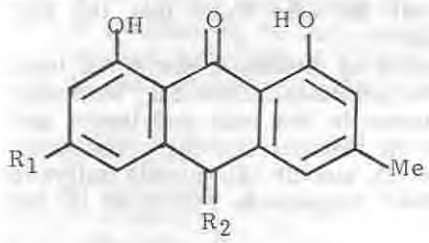
As part of our continuing phytochemical study of Ethiopian plants we have investigated the secondary metabolites of *R. prinoides*. Chemical screening showed the presence of traces of anthraquinones in the bark and leaves and copious amounts in the fruits. We report here the isolation and characterization of the known anthracene derivatives physcion (**1**), emodin (**2**), emodin anthrone (**3**), emodin bianthrone (**4**) and the new anthrone rhamnoside derivative (**5**) for which the trivial name prinoidin is proposed.

RESULTS AND DISCUSSION

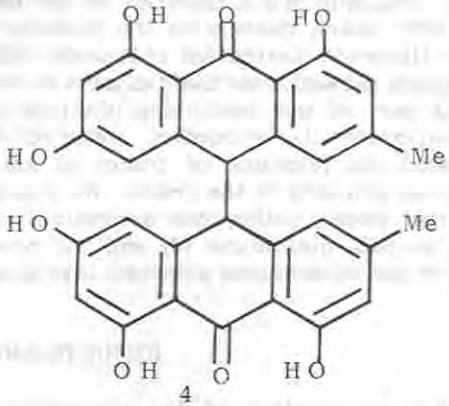
TLC examination of the chloroform extract of the fruits of *R. prinoides* indicated the presence of four anthracene derivatives in significant amounts and others in trace amounts. The crude extract was subjected to column chromatography on silica gel and eluted with chloroform/ethylacetate mixtures to yield fractions which were further chromatographed on micro columns to furnish the known compounds physcion (**1**), emodin (**2**) emodin anthrone (**3**) and emodin bianthrone (**4**). Physcion and emodin were readily identified by direct comparison with authentic specimens (co-tlc, mmp.). Compound **3** showed a parent ion peak at m/z 268 and in the IR spectrum an absorption band at 1630 cm^{-1} characteristic of only one doubly chelated carbonyl group was observed. The ^1H NMR spectrum displayed a singlet at 4.28 ppm indicative of the CH_2 group appropriate to an anthrone. Furthermore treatment of this compound

with trace of base caused its quantitative conversion to emodin. On the basis of these data compound **3** was identified as emodin anthrone. Compound **4** was identified by comparison of the acquired physical and spectroscopic data with those reported for emodin bianthrone by Cameron and coworkers (4). Interestingly emodin (**2**) and emodin bianthrone (**4**) were not present (tlc examination) in the original chloroform extract and are believed to be artefacts formed from emodin anthrone (**3**) upon chromatography on silica gel and by oxidation during workup respectively.

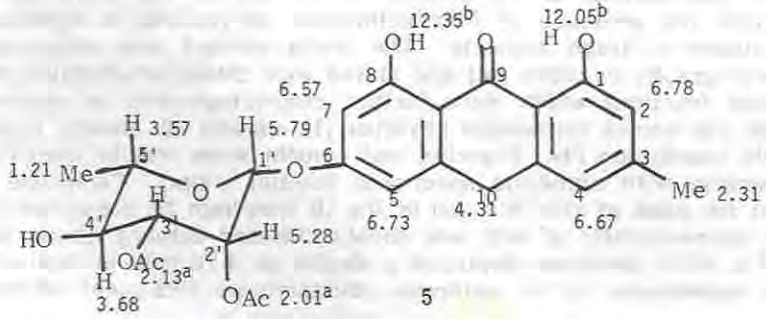
A portion of the residue obtained from the chloroform extract of the fruits was put on a column of acetylated polyamide and eluted with chloroform/hexane (1:2) to give a light orange solid, prinoidin (**5**). Prinoidin, m.p. 245-247°C, $[\alpha]_D$ -114° ($CHCl_3$, c = 0.076), HRMS 486.1523 suggested the molecular formula $C_{25}H_{26}O_{10}$. Upon acid hydrolysis it gave emodin anthrone (**3**) and L-rhamnose. The 400 MHz ^1H NMR spectrum showed that the sugar unit was attached to the oxygen at C-6, since all the protons of the aglycone part were accounted for except the replaced proton at C6-OH, usually observed at ca 10.2 ppm. The ^1H NMR, and the ^1H-^1H chemical shift correlated 2D (Cosy 45) spectra (see Fig. 1) were utilized to make assignments of the proton signals as well as to unequivocally determine the location of the two acetate units at C-2' and C-3'. The signal at 5.79 ppm (J = 2 Hz) is assignable to the acetal proton at 1' position of the sugar unit. This proton is coupled to H-2' which appears at a relatively low field (compared to H-4' and H-5') of 5.28 ppm (dd, J = 2, 4 Hz), suggesting that one of the acetate groups is located at C-2'. Similarly H-2' is coupled to H-3' at 5.09 ppm (J = 4, 9 Hz) which also occurs at low field suggesting attachement of the second acetate group to C-3'. Again H-3' couples



1 R_1 = OMe, R_2 = O
2 R_1 = OH, R_2 = O
3 R_1 = OH, R_2 = H,H



4



5

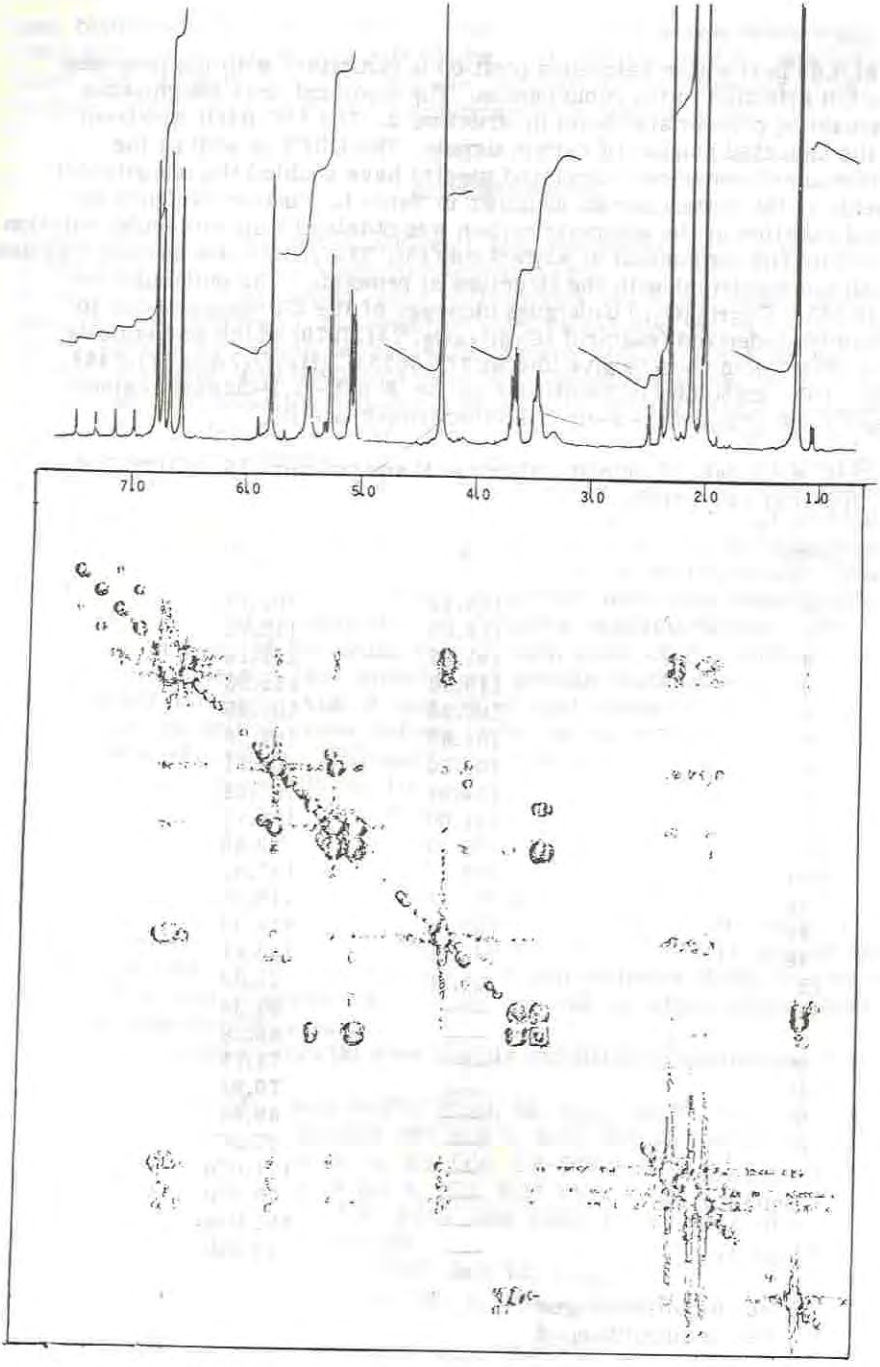


Fig. 1 $^{1}H - {}^{1}H$ Chemical shift correlated 2-D (Cosy 45) NMR of **5** at 360 MHz.

to H-4' at 3.68 ppm whose resonance position is consistent with the presence of a free OH attached to the same carbon. The chemical shift assignments of the remaining protons are shown in structure **5**. The ^{13}C NMR spectrum showed the expected number of carbon signals. The DEPT as well as the 2-dimensional heteronuclear correlated spectra have enabled the unequivocal assignments of the carbon signals as shown in Table 1. Further evidence for the α-configuration at the anomeric carbon was obtained from molecular rotation measurements and application of Klyne's rule (5). The HRMS also showed fragment ions which are consistent with the structure of prinoidin. The molecular ion (M^+ = 486.1523, $C_{25}H_{26}O_{10}$) undergoes cleavage of the C1'-oxygen bond to give a rhamnose-derived fragment ($C_{10}H_{15}O_6$, 231.0870) which sequentially loses two acetic acid units to give ions at 171.0655 ($C_8H_{11}O_4$) and 111.0448, $C_6H_7O_2$). Thus prinoidin is identified to be 6-0-L-[2,3-diacetylrhamnopyranosyl]-1,6,8-trihydroxy-3-methylanthracene-9-one (**5**).

Table 1 ^{13}C NMR data of emodin anthrone (**3**) and prinoidin (**5**) in DMSO-d_6 (ppm) at 100.6 MHz.

C-No	3	5
1	164.49	162.77
2	115.00	119.72
3	141.88	141.13
4	119.80	115.93
5	107.30	107.35
6	161.63	161.58
7	100.90	102.21
8	164.91	165.08
9	191.00	192.11
10	32.20	32.89
10a	146.95	147.56
8a	112.80	113.43
9a	108.30	111.34
4a	144.86	143.81
15	21.50	22.06
1'	—	95.34
2'	—	69.58
3'	—	71.73
4'	—	70.98
5'	—	69.84
6'	—	17.57
C=O(OAc)	—	171.07a
CH_3(OAc)	—	20.90b
C=O(OAc)	—	169.93a
CH_3(OAc)	—	20.80b

a - may be interchanged
b - may be interchanged

Physcion (**1**) and emodin (**2**) are common anthraquinones, frequently found in a number of plants including the family Rhamnaceae. Emodin anthrone (**3**)

and bianthrone (**4**) are known to occur in the well known crude plant purgative drug preparations of *R. frangula* (Alder Buckthorn) (6) and *R. purshiana* (Cascara bark) respectively (7). The emodin anthronediacetylrhamnoside (**5**) is a new natural product and the trivial name prinoidin is suggested for it.

EXPERIMENTAL

Plant material. The plant material was purchased from the medicinal plant market, Merkato, Addis Ababa in December 1985.

Apparatus. Melting points were determined on a hot stage Bock-Monoscop and are uncorrected. IR spectra were measured on a Perkin-Elmer 727 instrument. NMR spectra were measured using 360, and 400 MHz instruments. Optical rotations were measured using a Perkin-Elmer model 241 polarimeter.

Extraction and Isolation. Powdered fruits of *R. prinoides* (540 g) were extracted by maceration for 24 hrs with chloroform. Removal of the solvent yielded 52 g (9.6%) of crude extract, 7 g of which was applied on a column of 275 g silica gel 60 (Merck). Gradient elution with chloroform-ethyl acetate and monitoring the fractions with ready-made tlc plates (Si gel 60, F_{254}) yielded mixtures of anthraquinones which upon further purification on microcolumns of silica gel furnished physcion (**1**), followed by emodin anthrone (**3**), emodin (**2**) and emodin bianthrone (**4**). Other anthracene derivatives were also subsequently obtained but were too small in quantity for complete characterization. Tlc comparison of the components of the crude extract with those of the various fractions from the column indicated that emodin and emodin bianthrone were not present in the original mixture. Also it was noted that prinoidin which was clearly seen by tlc, in the initial crude extract, was not detected in any of the fractions eluted from the column. Prinoidin was however easily isolated on an acetylated polyamide column as follows: 1.0 g of the crude chloroform extract was dissolved in a minimum amount of a mixture of chloroform/ethyl acetate and applied on a 40 g of acetylated polyamide column and eluted with $CHCl_3$/hexane (1:1). 20 ml fractions were collected. The initial fractions contained physcion. Fractions 7-9 contained 47 mg of prinoidin.

Characterization of Anthracene Derivatives. The crude extract, upon tlc on silica gel and elution with toluene/EtOAc/HOAc (75:25:1), showed the presence of physcion (Rf = 0.88), emodin (0.49), emodin anthrone (0.46), emodin bianthrone (Rf = 0.27), and prinoidin (Rf = 0.25), as well as other minor pigments which have not been characterized.

Physcion (**1**) and emodin (**2**) were readily identified by comparison with authentic samples.

Emodin anthrone (3). M.p. 262°C (dec); IR, ν_{max} cm^{-1} (KBr): 3340, 1630, 1600, 1475; UV λ_{max} (MeOH) 267 (log ε, 3.8), 302 sh (3.9), 352 (4.2); ^{1}H NMR (DMSO-d_6) 12.35, 12.19 (s, s, C1-OH, C8-OH), 10.30 (s, C6-OH), 6.75 (br s, H-4), 6.65 (br s, H-2), 6.40 (br s, H5), 6.22 (d, J = 2.1 Hz, H-7), 4.28 (br s, H_2-10), 2.30 (br s, $ArCH_3$), ; ^{13}C NMR (see Table 1); MS, m/z (Rel. int): 256 (M^+, 100%), 241 (22), 227 (13), 223 (22).

Emodin bianthrone (4). M.p. >245° dec; IR, ν_{max} cm^{-1}(KBr): 3400, 1625, 1600, 1480, 1250; ^{1} HNMR see ref. 4. MS, m/z (rel. int): 257 (27), 256 (100), 255 (70), 227 (68).

Prinoidin (5). Light orange solid, m.p. 245-247°C. Optical rotation $[\alpha]^{21}$ (nm) (c = 0.076, $CHCl_3$): -114 (589), -119 (578), -139 (546), -1638 (436), -1671 (365); IR, ν_{max} cm^{-1} (KBr): 3680, 1740, 1630, 1600, 1500, 1250; UV λ_{max} ($CHCl_3$) 292 (log ε, 4.3), 346 (4.3); ^{1}H NMR see structure **5**; ^{13}C NMR see Table 1. MS, m/z (rel. int): 486.1523 (M^+, 6.2%) (calc. for $C_{25}H_{26}O_{10}$: 486.1526), 257 (14.9), 256 (41.5), 231 (60.1), 171 (100), 111 (40.9).

Hydrolysis of prinoidin **5**. A solution of prinoidin was prepared by dissolving 26 mg of **5** in 4 ml of ethanol to which were added 1.0 ml of water and 6 drops of conc HCl and refluxed for 2 hrs. The mixture was cooled and extracted with 4 ml of ethyl acetate, dried with anhydrous sodium sulphate and freed of solvent to give emodin anthrone identical in all respects with an authentic sample. The aqueous phase was concentrated, and analysed by paper chromatography using ethyl acetate/pyridine/water (12:5:4) as developing solvent. Detection with aniline hydrogen phthalate resulted in a brownish spot, identical in Rf and colour to that of authentic L-rhamnose.

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