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EMODIN AND EMODINANTHRONE RHAMNOSIDE ACETATES FROM FRUITS OF *RHAMNUS PRINOIDES*

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Key Word Index—*Rhamnus prinoides*; Rhamnaceae; fruits; anthraquinones; rhamnosides; flavonoid; rhamnazin.

Abstract—The fruits of *Rhamnus prinoides* yielded the known anthraquinones emodin and physcion, emodinanthrone, prinoidin, the flavonoid rhamnazin and five new pigments identified as acetylated derivatives of emodinanthrone and emodin rhamnopyranoside.

INTRODUCTION

Abegaz and Dagne [1] investigated the fruits of *Rhamnus prinoides* L'Herit and reported common anthraquinones, physcion, emodin, emodinanthrone, emodinbianthrone and a novel anthrone rhamnoside, prinoidin (**1**). These workers mentioned the presence of several trace pigments which were not characterized. We have not characterized five new pigments which are related to prinoidin.

RESULTS AND DISCUSSION

The residue from the chloroform extract of the defatted fruit powder, upon successive silica gel and Sephadex column chromatography sequentially yielded the pigments: **2**, prinoidin (**1**), physcion, emodin, rhamnazin (**8**), emodinanthrone, emodinbianthrone and **3**–**6**. Emodin, emodinanthrone, emodinbianthrone, physcion as well as the rhamnoside diacetate, prinoidin (**1**), previously reported from the same plant [1], were identified by direct comparison with authentic samples.

Compound **2**, mp 154–146° $[\alpha]_D^{21}$ − 61° ($CHCl_3$; *c* 0.1) had a ^{1}H NMR spectrum (Table 1) very similar to that of prinoidin (**1**) but differed in that all the hydroxyl groups of the sugar were acetylated and only a quartet of doublet signals of H-5′ of the rhamnose moiety was observed in the 3–4 ppm region. The presence of three acetate signals was indicated by the characteristic methyl resonance signals at δ 2.01, 2.11 and 2.22. The ^{13}C NMR and DEPT spectra data showed the expected number of quaternary, methine, methylene and methyl carbons (Table 2). This compound failed to show a molecular ion peak under EI-mass spectral conditions but showed a molecular ion at *m/z* 528 when the spectrum was determined under positive FAB ionization conditions. The IR spectrum showed absorption bands at 3468, 2917, 1755, 1660 (sh), 1626, 1598, 1217 cm^{-1}. The UV-vis spectrum was similar to that of emodinanthrone. Treatment of **2** with acid yielded emodinanthrone as the aglycone and rhamnose. The above data led to the conclusion that **2** is emodinanthrone-6-*O*-rhamnopyranoside-2′,3′4′-triacetate. Acetylation of prinoidin (**1**) was attempted to obtain the peracetate and to compare the NMR spectrum to that of **2**. Complete acetylation required up to one week at room temperature (see Experimental). Also, under the acetylation conditions, oxidation of the anthrone to anthraquinone occurred to yield **7**. The same peracetate was obtained by acetylation of **2**. This peracetate has been reported previously as an acetylation product of frangulin B obtained from *Rhamnus formosana* [2]. Only the ^{13}C NMR data were reported for frangulin B peracetate [2], and these were found to be similar to those measured by us.

Compound **3**, an orange-red substance, mp 230–232°, $[\alpha]_D^{21}$ − 84° ($CHCl_3$; *c* 0.14) showed an important difference in the ^{1}H NMR spectrum from those of **1** or **2** in having the H-4 and H-5 resonance signals considerably deshielded to δ 7.62 and 7.50, respectively, owing to the presence of the keto group at C-10. The sugar portion still contained two acetate groups and it was obvious that these were located at C-3′ and C-4′. This was concluded from the observation that the signal due to the acetal proton (δ5.7, *d*, *J* = 1.7 Hz, H-1′) was coupled to the signals of the equatorial H-2′ whose chemical shift at δ 4.28 is appropriate to the presence of a free hydroxyl group attached to the same carbon. The latter proton was coupled to H-3′, which, however, is located at δ5.40 (*dd*, *J* = 3.10 Hz) indicating that an acetate group is attached to C-3′. Likewise the H-3′ was coupled to H-4′ at δ5.19

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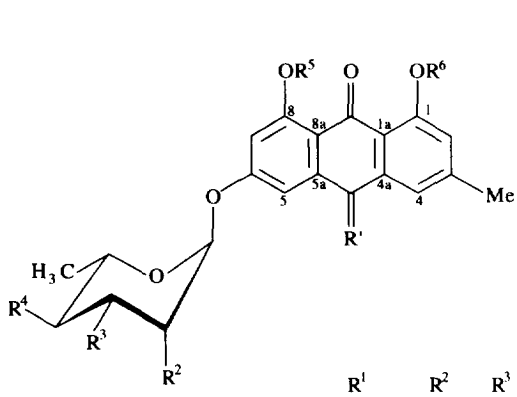

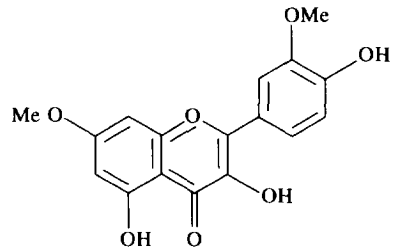

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	R^1	R^2	R^3	R^4	R^5	R^6
1	H,H	OAc	OAc	OH	H	H
2	H,H	OAc	OAc	OAc	H	H
3	O	OH	OAc	OAc	H	H
4	O	OAc	OAc	OH	H	H
5	O	OAc	OH	OAc	H	H
6	O	OAc	OH	OH	H	H
7	O	OAc	OAc	OAc	Ac	Ac

Table 1. ^{1}H NMR spectral data (400 MHz, $CDCl_3$) of compounds **1–6**

H	1[1]	2	3 (200 MHz)	4 (400 MHz)	5 (360 MHz)	6 (400 MHz)
2	6.78	6.76	7.08	6.91	7.08	7.05
4	6.67	6.67	7.62	7.48	7.63	7.58
5	6.73	6.75	7.50	7.62	7.45	7.39
7	6.57	6.57	6.93	7.07	6.89	6.86
10	4.31	4.25	—	—	—	
1′	5.79 *d* (2)	5.58 *d* (2)	5.69 *d* (1.7)	5.60 *d* (1.8)	5.69 *d* (1.7)	5.63
2′	5.28 *dd* (2, 4)	5.38 *m*	4.28 *br*	5.42 *dd* (1.8, 3)	5.24 *dd* (1.7, 4)	5.26
3′	5.09 *dd* (4, 9)	5.32 *m*	5.40 *dd* (3, 10)	5.31 *dd* (3, 11)	4.26 *m*	3.56
4′	3.68	5.18 *dd* (9, 10)	5.19 *dd* (10, 9)	3.75 *dd* (10,11)	4.95 *dd* (9.9, 9.7)	4.10
5′	3.57	3.76 *qd* (6, 9)	3.89 *m*	3.82 *qd* (6, 10)	3.88 *m*	3.74
6′	1.35 *d*	1.25 *d* (6)	1.19 *d* (6)	1.34 *d* (6)	1.21 *d* (6)	1.22
Me-Ar	2.31	2.36	2.44 *s*	2.44 *s*	2.45	2.42
OH-1,8	12.35, 12.05	12.22, 12.52	12.25, 12.03	12.24, 12.03	12.03, 12.24	12.00, 12.20
OAc	2.01, 2.13	2.01, 2.11, 2.22	2.04, 2.14	2.12, 2.22	2.12, 2.22	2.17

(*dd*, J = 9,10 Hz) indicating that the latter is attached to a carbon also carrying the second acetate group. This proton is further coupled to H-5′ at δ3.89. The two acetate methyls appeared at δ 2.04 and 2.14. Like **2** this compound also showed a molecular ion under positive FAB-mass spectral conditions at m/z 500. The IR spectrum showed the presence of an hydroxyl (3450 cm^{-1}) and carbonyl groups (1746, 1678 and 1629 cm^{-1}). The UV-vis spectrum was very similar to that of emodin.

Compound **4**, gum $[\alpha]_D^{21}$ − 56° ($CHCl_3$; c 0.14) showed ^{1}H and ^{13}C NMR spectra identical in the sugar portion to that of prinoidin and differed in the anthracene portion by showing a 9,10 quinone pattern as in **3**. The FAB-mass spectrum indicated the molecular ion at m/z 500. Like **3**, the IR spectrum showed an hydroxyl (3446 cm^{-1}) and carbonyl groups (1748, 1678 and 1614 cm^{-1}). The UV-vis spectrum was also similar to that of emodin. The presence of two acetate groups at the 2- and 3- positions of the sugar moiety was established following the same technique as described for **2**. Confirmation of this as well as the assignments of the proton signals were made by extensive decoupling of the various sugar signals at 400 MHz. This compound was found to be identical with a substance isolated in greater quantities from the leaves [3].

Compound **5**, mp 228–230°, $[\alpha]_D^{21}$ − 76° ($CHCl_3$; c 0.13), showed an R_f value very similar to that of **3** and posed considerable difficulties to achieve separation. The mixture of **5** and the isomeric **3** was separated on a preparative plate by multiple development using a chloroform–ethylacetate (9:1) solvent (50 ml) containing 10 drops of 5% HCl in methanol. Even then complete separation was not achieved and the ^{1}H NMR spectra of each of these showed small amounts of the other substance. The presence of two acetate groups at the 2- and 4-position of the rhamnose unit was easily established by analysis of the 200 MHz ^{1}H NMR spectrum. The FAB-

Table 2. ^{13}C NMR spectral data of compounds **1**–**7** (22.4 MHz, $CDCl_3$ for **1**–**5**, **7** and 75 MHz for **6***)

C	1	2	3	4	5	6	7
1	162.8	162.9	162.6	162.7	162.6	162.7	150.3
2	119.7	119.6	121.3	124.5	121.3	124.7	130.8
3	141.1	141.1	148.8	148.7	148.8	149.0	146.0
4	115.9	115.9	124.5	121.4	124.5	121.5	126.0
5	107.4	107.2	109.5	109.6	109.5	109.5	112.0
6	161.6	161.5	162.0	162.5	162.0	162.5	159.7
7	102.2	102.4	111.6	109.3	111.6	111.5	117.9
8	165.0	165.1	165.0	164.9	165.0	165.0	152.7
9	192.1	192.1	191.0	191.1	191.0	191.1	179.8
10	32.8	32.9	181.4	181.5	181.4	181.4	181.7
5a	147.6	147.5	135.5	133.3	135.5	135.5	136.3
8a	113.4	113.4	113.7	111.7	113.7	113.7	120.9
1a	111.3	111.5	109.3	113.7	109.3	109.4	123.7
4a	143.8	143.8	133.1	133.3	133.1	133.2	134.3
1′	95.3	95.4	97.4	95.7	95.4	95.6	95.7
2′	69.6	69.4	71.3	69.5	74.2	70.0	69.2
3′	71.7	70.6	70.9	71.7	72.0	73.1	68.7
4′	70.9	68.8	69.1	71.0	68.3	71.7	70.7
5′	69.8	67.7	67.9	70.3	67.7	69.7	68.1
6′	17.6	17.4	17.4	17.5	17.4	17.4	17.4
Ar-Me	22.0	21.9	22.1	22.1	22.1	22.3	21.6
CO-Me	171.1	169.8 × 3	169.9	170.9	171.3	170.9	169.8 169.3
	169.9		169.8	169.8	169.9	—	169.0
Me	20.8 20.9	20.6 × 3	20.6 20.8	20.7 20.8	20.6, 20.8	21.0	21.0 × 3, 20.7 × 3

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mass spectrum showed a molecular ion at m/z 500. The IR and UV spectra were similar to those of **3** and **4** (see Experimental).

Compound **6**, mp 123–125°, $[\alpha]_D^{21}$ −104° ($CHCl_3$; c 0.14). The ^1H NMR spectrum clearly showed the presence of only one acetate group at δ2.17. H-1′ of the sugar appeared at δ5.65 and this was coupled to a proton attached to an acetate-bearing carbon at δ5.26. The absence of any other signal in the δ4.5–5.5 region confirmed that there was only one acetate group on the sugar residue. The interrelationships of the other signals were determined from the H–H COSY spectrum at 400 MHz. The IR, UV and ^{13}C NMR spectra (see Experimental) unequivocally established the structure as **6**.

A fairly polar compound was also isolated which was identified from the ^1H NMR as the flavonoid rhamnazin, previously isolated from *R. lycioides* [4] and *R. alaternus* [5]. The identity of this substance was established from HRMS data as well as by comparison of acquired IR, UV and NMR data with data reported in the literature [4–6].

The occurrence of 6-*O*-rhamnosides of emodin has been reported on several occasions from various taxa belonging to *Rhamnus*. Examples include frangulin A, B and glucofrangulin A from *R. fallax* [7] and *R. procumbens* [8, 9]. However, acetylated rhamnosides are rather rare. The possibility that these acetates may be artifacts was ruled out since these compounds were still found even after care has been exercised to exclude solvents and conditions that would result in ester exchange. It is not possible to rule out the occurrence of intramolecular trans-esterification which may in principle yield **3** from **5** or vice versa, although there was no positive evidence that this was happening.

EXPERIMENTAL

General. Mp: uncorr. FT-IR KBr discs. ^1H NMR at 200, 360 or 400 MHz; ^{13}C NMR at 22.4 MHz. ^1H and ^{13}C chemical shifts are referenced to the residual $CHCl_3$ (δ7.26), $CDCl_3$ (δ77.0) signals, respectively. Analytical TLC: silica gel (Merck, Kiesel gel 60_{254}, 0.25 mm), flash CC: silica gel (Merck 9385 Kiesel gel 60 particle size 0.040–0.063 m, impregnated with 5% aq. oxalic acid). Prep. TLC: silica gel (Merck 7748, Kiesel gel 60 $PF_{254+366}$, 1 mm). Spots were visualized with 3% ethanolic KOH.

Plant material. The fruits of *Rhamnus prinoides* L'Herit were purchased from a vendor in the Central Market (Merkato) in Addis Ababa.

Extraction and separation. The dried and powdered fruits (1 kg) were defatted with petrol followed by extraction with $CHCl_3$ to yield a residue of 52 g. A portion of this residue (15 g) was applied on 300 g flash grade silica gel and eluted with $CHCl_3$ and 19 × 250 ml frs were collected. Further elution (frs 20–24) was made using $CHCl_3$–EtOAc (4:1) followed by (frs 25–28) $CHCl_3$–EtOAc (1:1). Based on TLC examinations the following fractions were combined: 3–5 (Fr. A), 6 (Fr. B), 7–19 (Fr. C), 21–22 (Fr. D mostly emodinanthrone, and was not worked on further) and 23–28 (Fr. E).

Fraction A. Introduced on to a Sephadex LH-20 column equilibrated with $CHCl_3$–MeOH (2:1). Twelve fractions (100 ml each) were collected. The third fr. yielded 83 mg of impure **2**, which was purified by passing through a micro-column of flash grade silica gel (30 g) and eluting with $CHCl_3$–EtOAc (9:1) to yield 10 mg pure **2**, mp 154–156°, $[\alpha]_D^{21}$ −61 ($CHCl_3$; *c* 0.1); IR ν_{max}^{KBr} cm^{-1}: 3468, 2917, 1755, 1660, 1626, 1598, 1217. UV-vis λ_{max}^{EtOH} nm: 272, 298, 353; FAB-MS (+ve mode) indicated a molecular ion at 529 $[M+H]^+$ consistent with $C_{27}H_{28}O_{11}$ with additional ions at 413, 256 $[M-\text{sugar}+H]^+$ and 42 (100%). ^1H NMR (200 MHz, $CDCl_3$: see Table 1); ^{13}C NMR (22.4 MHz, $CDCl_3$: see Table 2). The fourth fr. from the above Sephadex column gave 150 mg of a mixture of **2** and prinoidin, fr. 5 yielded pure prinoidin (40 mg) identical in all respects, mp, mmp, IR, ^1H NMR to those reported in the literature [1]. Frs 6–9 gave 400 mg physcion. Frs 10–11 gave a mixture of emodin and rhamnazin. Fr. 12 gave pure rhamnazin, mp 213–215°, IR ν_{max}^{KBr} cm^{-1}: 3472, 3288, 1655, 1592, 1505, 1156. UV λ_{max}^{EtOH} nm: 206, 255, 372, + $AlCl_3$, 211, 267, 428, ^1H NMR (200 MHz, DMSO-d_6): δ 3.85 (3H, *s*, OMe), 3.86 (3H, *s*, OMe), 6.34 (*d*, 1H, *J* = 2.2 Hz, H-5), 6.79 (*d*, 1H, *J* = 2.2 Hz, H-7), 6.94 (*d*, 1H, *J* = 8.2 Hz, H-5′), 7.75 (*dd*, 1H, *J* = 8.2, 2.2 Hz, H-6′), 7.7 (*d*, 1H, *J* = 2.2 Hz, H-2′), 9.61 (OH), (9.65 (OH) and 12.46 (OH), HR-MS $C_{17}H_{14}O_7$ 330.0738, calc. 330.0739.

Fraction B. Contained mostly **2**. This fraction (300 mg) was not worked on further.

Fraction C. The $CHCl_3$-insoluble portion was found to be emodinanthrone. The soluble portion was applied on Sephadex LH-20 ($CHCl_3$–MeOH, 2:1) and the post-chlorophyll portion, first yielded **1**, and **3** which seemed to be one compound at first but was found to be a mixture, see below, followed by a mixture of **3** and emodinanthrone. Compound **3** was purified by prep. TLC (multiple development with 10 ml $CHCl_3$–EtOAc 4:1 containing 5 drops of 5% HCl in MeOH) to give **4**, (4 mg), $[\alpha]_D^{21}$ −56° ($CHCl_3$; *c* 0.13); IR ν_{max}^{KBr} cm^{-1}: 3446, 2920, 1748, 1678, 1614, 1260, 1033; UV-vis λ_{max}^{EtOH} nm: 263, 289, 297, 433; only FAB-MS (+ve mode) indicated a molecular ion at 500, consistent with $C_{25}H_{24}O_{11}$; ^1H NMR (400 MHz, $CDCl_3$: see Table 1); ^{13}C NMR (22.4 MHz, $CDCl_3$: see Table 2); **3**, 40 mg (again not pure, but containing **5**) and 5 mg of **5**. The impure **3** (40 mg) was applied on analytical pre-coated TLC-plate and multiple development with $CHCl_3$–EtOAc (9:1, 50 ml) containing 10 drops of 5% HCl in MeOH. This gave **3**, 17 mg mp 230–232°, $[\alpha]_D^{21}$ −84° ($CHCl_3$; *c* 0.14); IR ν_{max}^{KBr} cm^{-1}: 3450, 2923, 1746, 1678, 1629, 1257, 1214; UV-vis λ_{max}^{EtOH} nm: 266, 298, 435; only FAB-MS (+ve mode) indicated a molecular ion at 500 consistent with $C_{25}H_{24}O_{11}$; ^1H NMR (200 MHz, $CDCl_3$: see Table 1); ^{13}C NMR (22.4 MHz, $CDCl_3$: see Table 2); and **5**, 14 mg, mp 228–230°, $[\alpha]_D^{21}$ −76° ($CHCl_3$; *c* 0.13); IR ν_{max}^{KBr} cm^{-1}: 3416, 2927, 1734, 1679, 1627, 1262, 1214; UV-vis λ_{max}^{EtOH} nm: 265, 291, 435; only FAB-MS (+ve mode) indicated a molecular ion at 500 consistent with $C_{25}H_{24}O_{11}$; ^1H NMR (200 MHz, $CDCl_3$: see Table 1); ^{13}C NMR (22.4 MHz, $CDCl_3$: see Table 2).

Fraction E. Applied on a chromatotron plate and eluted with $CHCl_3$–EtOAc (4:1) and 20 × 10 ml frs were collected. Frs 4–6 contained a mixture of dimeric compounds which could not be separated and were not examined further. Frs 14–20 yielded 30 mg of **6**, mp 123–125°, ν_{max}^{KBr} cm^{-1}: 3411, 2924, 2852, 1746, 1631, 1260, 1216, 975, 758; λ_{max}^{EtOH} nm: 221, 327, 445. ^1H NMR (see Table 1); ^{13}C NMR (see Table 2).

Acetylation of **1** *and* **2**. Compound **1** (100 mg) dissolved in 10 ml Ac_2O and 3 drops of pyridine was stirred for two days. The mixture was poured into 50 g of crushed ice and stirred for 5 hr. Extraction with $CHCl_3$, gave a residue which showed five spots showing incomplete levels of acetylation. Reacetylation of the mixture of products resulted in the formation of the maximum amount of the peracetate. The peracetate was purified by prep. TLC ($CHCl_3$–EtOAc 4:1), 28 mg, mp 121–122°, ν_{max}^{KBr} cm^{-1}: 3021, 1750, 1672, 1603, 1521, 1206, 774; λ_{max}^{EtOH} nm: 223, 267, 288, 300, 347; only FAB-MS (+ve mode) indicated a molecular ion at 626 consistent with $C_{31}H_{30}O_{14}$; ^1H NMR (200 MHz, $CDCl_3$: δ 7.20 (*d*, *J* = 2 Hz, 1H, H-2), 7.95 (*br*, 1H, H-4), 7.85 (*d*, *J* = 2 Hz, 1H, H-5), 5.65 (*d*, *J* = 2 Hz, 1H, H-1′), 5.41 (*m*, 2H, H-2′ and H-3′), 5.20 (*dd*, *J* = 9, 10 Hz, H-4′), 3.89 (*dd*, *J* = 6,9 Hz, H-5′), 1.20 (*d*, *J* = 6 Hz, 3H, Me-6′), 2.49 (*s*, 3H, Me-3), 2.01, 2.11, 2.22 (3 × OAc); ^{13}C NMR (22.4 MHz, $CDCl_3$: see Table 2). Acetylation of **2** was carried out in the same manner and eventually yielded a peracetate identical to the one obtained from **1**.

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REFERENCES

1. Abegaz, B. and Dagne, E. (1988) *Bull. Chem. Soc. Ethiop.* **2**, 15.
2. Lin, C.-N., Chung, M.-I. and Lu, C.-M. (1990) *Phytochemistry* **29**, 3903.
3. Kebede, T. (1994) M.Sc. Dissertation, Addis Ababa University.
4. Payá, M., Máñez, S. and Villar, A. (1986) *Z. Naturforschung.* **41c**, 976.
5. Riess-Maurer, I. and Wagner, H. (1982) *Tetrahedron* **38**, 1269.
6. Valesi, G., Rodriguez, E., Vander Velde, G. and Mabry, T. J. (1972) *Phytochemistry* **11**, 2821.
7. Kinget, R. (1967) *Planta Med.* **15**, 223.
8. Majumder, P. L. and Chattopadhyay, A. (1985) *J. Indian Chem. Soc.* **62**, 616.
9. Dwivedi, S. P. D., Pandey, V. B., Shah, A. H. and Rao, Y. B. (1988) *Phytotherapy Res.* **2**, 51.