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TRITERPENOIDS FROM *MYRICA RUBRA**

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Key Word Index *Myrica rubra*; Myricaceae; taraxerone; taraxerol; myricadiol; 28-hydroxy-D-friedoolean-14-en-3-one.

Abstract—A new triterpene has been isolated together with taraxerone, taraxerol, myricadiol and sitosterol from the stem bark of *Myrica rubra*. On the basis of chemical and spectral evidence, the structure was established as 28-hydroxy-D-friedoolean-14-en-3-one.

INTRODUCTION

The bark of *Myrica rubra* Sieb. et Zucc. has been used in Japan and China as an astringent, an antidote and as an antidiarrhoea agent.

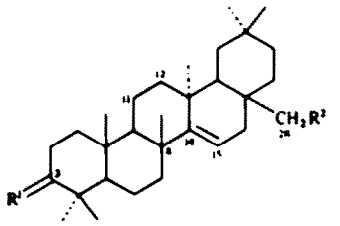
Recently, diarylheptanoid [1], a galloyl flavanonol sulphate, tannin and related compounds [2] were isolated from the stem bark of *M. rubra*. We have examined the same source and report the isolation and characterization of a new triterpenoid together with taraxerone, taraxerol, myricadiol and sitosterol.

RESULTS AND DISCUSSION

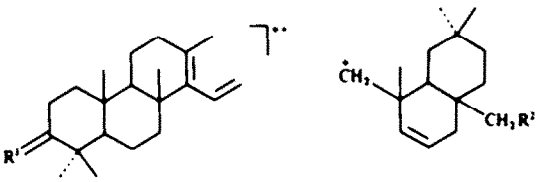
The benzene extract of the stem bark was separated into acidic and neutral fractions, and compounds **1**–**5** were isolated from the neutral fraction. Compound **1**, $C_{30}H_{48}O$, mp 240–243°, gave a mass spectrum which showed intense peaks at *m/z* 300 (ion a) and 204 (ion b) (Scheme 1). Compound **1** was identified as taraxerone by comparison with an authentic sample [3]. Compound **2**, $C_{30}H_{50}O$, mp 278–279°, gave a mass spectrum which showed intense peaks at *m/z* 302 (ion c) and 204 (ion b). Compound **2** was identical with an authentic sample of taraxerol [4]. Compound **3**, mp 137–140°, was identified as sitosterol by its spectral data.

Compound **4**, $C_{30}H_{50}O_2$, mp 271–272°, gave a mass spectrum which showed intense peaks at *m/z* 302 (ion c) and 220 (ion d). Acetylation of **4** with acetic anhydride in pyridine afforded a diacetate (**4a**), $C_{34}H_{54}O_4$, mp 259–260°, and a monoacetate (**4b**), $C_{32}H_{52}O_3$, mp 249–250°. The IR and ^{1}H NMR spectral data of **4a** were in good agreement with those of an authentic sample of myricadiol diacetate [4] and thus compound **4** was identified as myricadiol.

Compound **5**, mp 225–227°, $[\alpha]_D$ −0.2°, analysed for $C_{30}H_{48}O_2$ and its IR spectrum showed hydroxyl (3350, 1000 cm^{-1}) and carbonyl (1708 cm^{-1}) absorption. Its ^{1}H NMR spectrum exhibited a double doublet of an olefinic proton at δ5.54 (J = 8.1 and 3.4 Hz) and two AB-type doublets at δ3.14 and 3.30 (J = 10.0 Hz) due to a hydroxymethylene group. In the mass spectrum of **5**, strong peaks at *m/z* 300 (ion a), 220 (ion d) and 189 (ion d $-CH_2OH$) indicated the presence of a double bond at C-14 and the hydroxymethylene group at C-28 having no substituents in rings C, D and E. Acetylation of **5** afforded a monoacetate (**5a**), $C_{32}H_{50}O_3$, mp 210–211°. From the above result, we can deduce that compound **5** is 28-hydroxy-D-friedoolean-14-ene with a carbonyl group probably at C-3.



1 R¹ = O, R² = H
2 R¹ = α-H, β-OH, R² = H
4 R¹ = α-H, β-OH, R² = OH
4a R¹ = α-H, β-OAc, R² = OAc
4b R¹ = α-H, β-OH, R² = OAc
5 R¹ = O, R² = OH
5a R¹ = O, R² = OAc



ion a *m/z* 300, R¹ = O
ion b *m/z* 204, R² = H
ion c *m/z* 302, R¹ = α-H, β-OH
ion d *m/z* 220, R² = OH
ion e *m/z* 344, R¹ = α-H, β-OAc
ion f *m/z* 262, R² = OAc

Scheme 1. Mass spectral fragments derived from compounds **1**, **2**, **4** and **5**.

*Part 2 in the series "Constituents of *Myrica rubra*". For Part 1, see ref. [1].

The skeletal structure was confirmed by chemically relating myricadiol (4) to compound 5. Oxidation of myricadiol monoacetate (4b) with chromium trioxide–pyridine gave the monoacetate (5a). Deacetylation of 5a afforded 28-hydroxy-D-friedooolean-14-en-3-one, which was identical with compound 5.

The ^{13}C NMR chemical shifts of compounds 1–5 were assigned by comparison with the reported shifts of aleuritolonic acid (6) and acetoxyaleuritolate (7) [5], and are shown in Table 1. In the ^{13}C NMR spectrum of 5, all signals were in good agreement with the proposed structure.

Taraxerone, taraxerol and myricadiol were isolated from the root bark of *M. cerifera* [3], taraxerol and myricadiol from the bark of *M. esculenta* [4], and myricadiol from the stem bark of *M. gale* [6] and *M. nagi* [7]. Taraxerol was also found in the leaves of *M. rubra* [8]. From the chemotaxonomic view, these triterpenoids seem to be widely distributed in *Myrica* species.

EXPERIMENTAL

All mps were uncorr. ORDs were measured using a 1 dm cell. ^1H NMR spectra were taken at 100 MHz in $CDCl_3$ soln using TMS as internal standard. ^{13}C NMR spectra were recorded at 25 MHz in $CDCl_3$ (TMS as internal standard). MS were run on a double focusing mass spectrometer (accelerating voltage of 3.0–6.5 kV; ionizing potential 70 eV). TLC was carried out on silica gel.

Extraction and isolation of constituents. Air-dried and ground stem bark of *M. rubra* (4 kg) was extracted with C_6H_6. The C_6H_6 soln was concd *in vacuo* to 2 l., and 2 M NaOH was added to the extract. The NaOH soln was partitioned with EtOAc. The EtOAc extract (20.0 g) was chromatographed repeatedly on silica gel using solvent systems of C_6H_6–EtOAc and $CHCl_3$–MeOH to give 1 (560 mg), 2 (2.7 g), 3 (530 mg), 4 (2.7 g) and 5 (26 mg).

Taraxerone (1). Colourless plates, mp 240–243° ($CHCl_3$–MeOH) (lit. [3] 238–239°). $[\alpha]_D^{27}$ + 9.6° ($CHCl_3$; *c* 1.01). Taraxerone (1) was identified by direct comparison with an authentic sample (mmp, TLC, ^1H NMR). ^{13}C NMR: see Table 1.

Taraxerol (2). Colourless needles, mp 278–279° (C_6H_6) (lit. [4] 273–274°). $[\alpha]_D^{27}$ + 0.5° ($CHCl_3$; *c* 1.10). Taraxerol (2) was identified by direct comparison with an authentic sample (mmp, TLC, ^1H NMR). ^{13}C NMR: see Table 1.

Sitosterol (3). Colourless needles, mp 137–140° (EtOH) (lit. [9] 138–139°). Sitosterol (3) was identified by direct comparison with an authentic sample (TLC, IR).

Myricadiol (4). Colourless needles, mp 271–272° ($CHCl_3$–MeOH) (lit. [4] 268–269°). ^{13}C NMR: see Table 1.

Table 1. ^{13}C NMR chemical shifts of taraxerone (1), taraxerol (2), myricadiol (4), compound 5, the acetate of 5 (5a), aleuritolonic acid (6) and acetoxyaleuritolate (7)

Carbon No.	1	2	4	5	5a	6	7
C-1	38.4	38.1	37.8	38.3	38.4	38.4	37.4
C-2	34.1	27.3	28.0	34.0	34.1	34.1	23.4
C-3	217.3	79.2	78.2	217.2	217.2	217.3	80.8
C-4	47.6	39.1	41.4	47.5	47.6	47.5	37.6
C-5	55.8	55.7	56.0	55.7	55.8	55.7	55.6
C-6	20.0	19.0	19.2	19.9	19.9	21.5	18.7
C-7	35.2	35.3	36.3	35.8	35.7	35.4	35.3
C-8	38.9	38.9	39.3	39.0	39.0	38.9	39.0
C-9	48.7	48.9	45.6	48.6	48.6	48.6	49.0
C-10	37.6	37.9	37.8	37.5	37.4	37.3	37.3
C-11	17.5	17.7	17.9	17.3	17.3	17.3	17.3
C-12	35.8*	35.9*	31.2*	30.7*	31.0*	31.3*	31.2
C-13	37.7	37.9	38.3	37.7	37.7	37.8	37.9
C-14	157.6	158.1	158.7	158.5	158.3	162.1	160.5
C-15	117.2	117.0	116.8	116.0	116.2	117.1	116.8
C-16	36.7*	36.9*	33.2*	32.6*	32.6*	30.7	30.6
C-17	37.7	38.1	38.3	40.3	39.0	51.5	51.5
C-18	48.8	49.4	49.6	44.9	44.6	41.4	41.3
C-19	40.7	41.4	41.7	40.6	40.6	40.3	40.7
C-20	28.8	29.0	28.8	28.5	28.5	29.3	29.3
C-21	33.6	33.9	33.8	33.3	33.5	33.7	33.6
C-22	33.1	33.2	28.7	27.9	28.3	31.9*	31.8
C-23	26.2	28.1	28.4	26.1	26.1	26.1	27.9
C-24	21.5	15.6	16.5	21.6	21.8	20.0	16.6
C-25	14.8	15.6	15.7	14.8	14.8	15.0	15.7
C-26	29.9	30.1	30.1	29.8	29.8	28.7	28.6
C-27	25.6	26.0	26.2	25.7	25.7	25.9	26.2
C-28	29.9	30.1	64.6	65.4	65.9	184.7	184.4
C-29	33.4	33.5	33.8	33.5	33.3	33.2	33.3
C-30	21.4	21.5	22.0	21.4	21.5	22.6	22.4

*Values in any vertical column may be reversed although those given here are preferred.

Acetylation of **4**. A soln of **4** (200 mg) in pyridine (50 ml) was acetylated with Ac_2O (0.09 ml) at room temp. for 30 hr. After usual work-up, the crude product was chromatographed on silica gel to give the diacetate (**4a**, 10 mg) and the monoacetate (**4b**), 98 mg). Diacetate of **4** (**4a**), colourless needles, mp 259 260° (EtOH) (lit. [4] 252–253°), $[\alpha]_D^{23}$ + 6.9° ($CHCl_3$; *c* 1.00). The diacetate of **4** (**4a**) was identified by direct comparison with an authentic sample (mmp, TLC, IR). Monoacetate of **4** (**4b**), colourless needles, mp 249 250° ($CHCl_3$–EtOH), $[\alpha]_D^{25}$ + 3.0° ($CHCl_3$; *c* 1.01). (Found: C, 79.28; H, 10.93. $C_{32}H_{52}O_3$ requires: C, 79.28; H, 10.81%.) IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620, 1020 (OH), 1730, 1220 (OAc). ^{1}H NMR: δ0.80, 0.90, 0.92 (3H each), 0.96 (6H), 0.97, 1.08 (3H each) (all *s*, Me × 7), 2.03 (3H, *s*, OAc), 3.18 (1H, *m*, H-3), 3.72 (2H, *s*, H_2-28), 5.45 (1H, *dd*, *J* = 8.2, 3.7 Hz, H-15). MS *m/z*: 484 $[M]^+$, 466 $[M - H_2O]^+$, 302 (ion c), 262 (ion f), 202 (ion f – HOAc), 189 (base peak) (ion f – CH_2OAc).

28-Hydroxy-D-friedoolean-14-en-3-one (**5**). Colourless needles, mp 225 227° ($CHCl_3$), $[\alpha]_D^{20}$ – 0.2° ($CHCl_3$; *c* 1.00). IR ν_{max}^{KBr} cm^{-1}: 3350, 1080 (OH), 1708 (CO), 1640 (C=C). ^{1}H NMR: δ0.90 (3H), 0.97 (6H), 1.07 (3H), 1.08 (6H), 1.11 (3H) (all *s*, Me × 7), 2.32–2.62 (2H, *m*, H_2-2), 3.14, 3.30 (1H each, *d*, *J* = 10.0 Hz, H_2-28), 5.54 (1H, *dd*, *J* = 8.1, 3.4 Hz, H-15). MS *m/z*: 440.3657 $[M]^+$ (calc. for $C_{30}H_{48}O_2$, 440.3642), 425 $[M - Me]^+$, 409 $[M - CH_2OH]^+$, 300 (ion a), 220 (ion d), 189 (base peak) (ion d – CH_2OH). Compound **5** was identified by comparison with an authentic sample derived from myricadiol (**4**) (mmp, TLC, ^{1}H NMR, IR). ^{13}C NMR: see Table 1.

Acetylation of **5**. A soln of **5** (5 mg) in pyridine (1 ml) was acetylated with Ac_2O (3.5 μl) at room temp. overnight. After usual work-up, the crude product was chromatographed on silica gel to give a monoacetate (**5a**) (4.0 mg), colourless needles, mp 210 211° (EtOH), $[\alpha]_D^{22}$ + 8.4° ($CHCl_3$; *c* 0.98). (Found: C, 79.43; H, 10.69. $C_{32}H_{50}O_3$ requires: C, 79.62; H, 10.44%.) IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1739, 1235 (OAc), 1705 (CO). ^{1}H NMR: δ0.90 (3H), 0.96 (6H), 1.06 (3H), 1.08 (6H), 1.13 (3H) (all *s*, Me × 7), 2.03 (3H, *s*, OAc), 2.30–2.62 (2H, *m*, H_2-2), 3.73 (2H, *s*, H_2-28), 5.47 (1H, *dd*, *J* = 8.0, 3.0 Hz, H-15). MS *m/z*: 482 $[M]^+$, 422 $[M - HOAc]^+$, 409 $[M - CH_2OAc]^+$, 300 (ion a), 262 (ion f), 202 (ion f – HOAc), 189 (base peak) (ion f – CH_2OAc). ^{13}C NMR: see Table 1.

Oxidation of **4b**. A soln of **4b** (163 mg) in pyridine (20 ml) was oxidized with CrO_3 (100 mg) at room temp. overnight. After work-up in the usual manner, the crude product was chromatographed on silica gel to give the manoacetate (**5a**, 135 mg), colourless needles, mp 210–211° (EtOH); MS *m/z*: 482.3767 $[M]^+$ (calc. for $C_{32}H_{50}O_3$, 482.3762).

Alkaline hydrolysis of **5a**. To a soln of compound **5a** (70 mg) derived from **4b** in EtOH (15 ml) was added 1.7% NaOEt EtOH (2 ml) and the soln was allowed to stand at room temp. overnight. The solvent was removed and the residue was extracted with $CHCl_3$, which was washed with 0.1 N H_2SO_4 and H_2O and then dried (Na_2SO_4). After evaporation of the solvent, the residue was chromatographed on silica gel to give compound **5** (67 mg), colourless needles, mp 224 225° ($CHCl_3$).

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