

THE FIRST ISOLATION OF LIGNANS, MEGACEROTONIC ACID AND  
ANTHOCEROTONIC ACID, FROM NON-VASCULAR PLANTS,  
ANTHOCEROTAE (HORNWORTS)

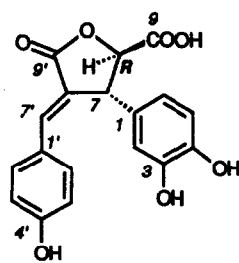
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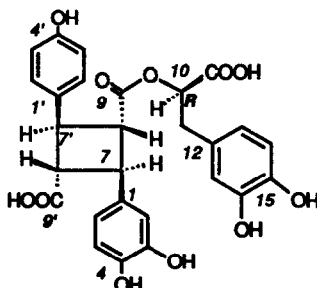
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**SUMMARY:** Two new lignans, megacerotonic acid and anthocerotonic acid, were isolated from the Anthocerotae (Hornworts), *Megaceros flagellaris* and *Anthoceros punctatus*. Their structures were determined by spectroscopic methods. This is the first known example of lignans isolated from non-vascular plants.

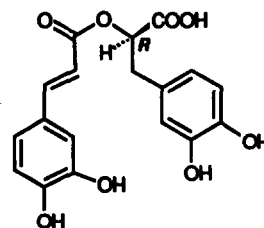
The bryophytes are non-vascular plants taxonomically placed between algae and pteridophytes. They are morphologically divided into three classes, mosses, liverworts and hornworts. In general, the vascular plants biosynthesize lignans and lignins in order to form the vascular bundles, while non-vascular plants don't produce such kinds of phenolic compounds. The isolation of lignans from non-vascular plants has not been reported. In the course of our investigations on secondary metabolites of bryophytes<sup>1</sup>, we investigated the secondary constituents of two species, *Megaceros flagellaris* and *Anthoceros punctatus*, belonging to the Anthocerotae (Hornworts). We now report the isolation and structural determination of two lignans named megacerotonic acid (1) and anthocerotonic acid (2). This is the first known



megacerotonic acid 1



anthocerotonic acid 2

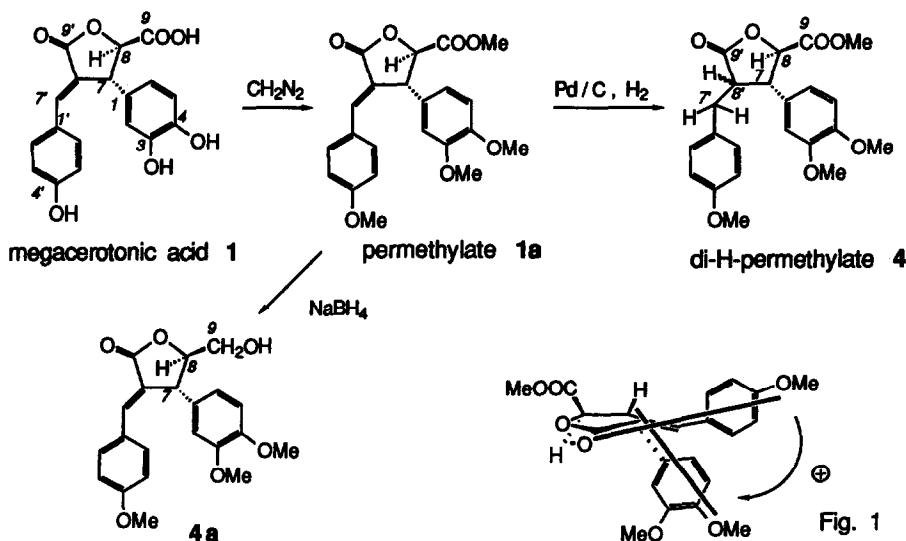


(R)-rosmarinic acid 3

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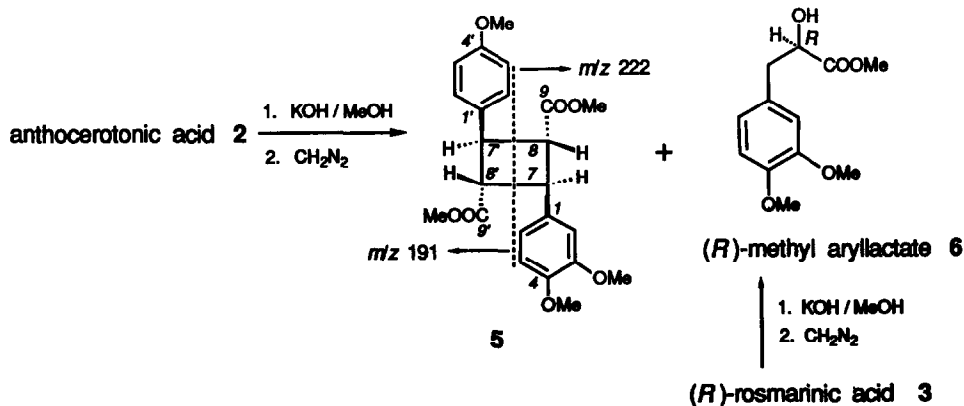
example of lignans isolated from non-vascular plants. Megacerotic acid (**1**) (100 mg) was isolated from 2% acetic acid extracts of fresh material (100 g) of *Megaceros flagellaris* collected in Kyoto Prefecture, by HPLC on M & S gel using 25% MeOH, 0.1% BuOH and 2% AcOH in H<sub>2</sub>O, while **2** (10 mg) together with rosmarinic acid **3**<sup>2</sup> and caffeic acid were isolated from 2% acetic acid extracts of fresh material (100 g) of *Anthoceros punctatus* collected in Kyoto Prefecture by a similar procedure.

The <sup>1</sup>H and <sup>13</sup>C NMR data of **13** indicated the presence of *m*, *p*- and *p*-substituted phenyl rings. Megacerotic acid was methylated with CH<sub>2</sub>N<sub>2</sub> in MeOH to yield **1a**, <sup>1</sup>H NMR of which showed signals of four methyl groups. Hydrogenation of **1a** with 10% Pd/C in MeOH yielded dihydro compounds **4** (*m/z* 400), IR spectra of which showed a  $\gamma$ -lactone absorption band at 1780 cm<sup>-1</sup>. The proton system comprising carbons 7', 8', 7 and 8 was elucidated by decoupling experiments on **4**. Also, **1a** was treated with NaBH<sub>4</sub> in MeOH to give **4a**, the IR spectrum of which indicated the presence of an  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone at 1645 and 1745 cm<sup>-1</sup>. In the <sup>1</sup>H NMR spectrum, 8-H was observed as a double of triplet (4.45 ppm, *J* = 3.8, 4.9 Hz) instead of a doublet in **1a**. This fact indicates that the -CH<sub>2</sub>OH group must be attached to C<sub>8</sub>. The stereochemistry and the position of the phenyl rings were determined by NOE experiments; an NOE was observed between the vinyl proton at C<sub>7'</sub> and proton(s) at C<sub>2'</sub> or/and C<sub>6'</sub> on the *p*-substituted phenyl ring. This observation indicates that the *p*-substituted phenyl ring is attached to C<sub>7'</sub>. A further NOE was observed between 7-H and proton(s) at C<sub>2'</sub> or/and



C<sub>6</sub> indicating the geometry of the vinyl group is *E*. An NOE was also observed between 8-H and 2-H indicating that 2-H and the *m,p*-substituted phenyl ring has a *cis* relationship. The absolute configuration was determined by the CD exciton chirality method<sup>4</sup>. The CD spectrum of **1a** shows positive Cotton effects at 305 nm,  $\Delta\epsilon_{305} +8.5$  and 232 nm,  $\Delta\epsilon_{232} -11.7$ . This establishes the chirality of the *m, p*-substituted phenyl group at C<sub>7</sub> and *p*-coumaran moiety as positive (Fig. 1), and the absolute configuration of megacerotonic acid as depicted. All these data show that the new lignan is most favourably represented by formula **1**.

The spectral properties of anthocerotonic acid (**2**)<sup>5</sup> indicated the presence of two *m, p*- and one *p*-substituted phenyl rings and an ester group. Methylation of **2** with CH<sub>2</sub>N<sub>2</sub> furnished a permethyl derivative. The proton system involving carbons 7, 8, 7' and 8' was elucidated by decoupling experiments on the permethyl derivative. These protons must be attached to a cyclobutane ring and are *trans* to each other as indicated by the coupling constants ( $J = 9.8$  Hz, respectively). Treatment with 5% KOH-MeOH followed by methylation with CH<sub>2</sub>N<sub>2</sub> gave **5** and **6**. An alcohol part **6** was identified as methyl aryllactate<sup>2</sup> which was also derived from (*R*)-rosmarinic acid **3** on treatment with 5% KOH-MeOH and CH<sub>2</sub>N<sub>2</sub>. The *R* configuration at C<sub>7</sub> was determined by comparison of the CD spectrum of (*R*)-methyl aryllactate derived from (*R*)-rosmarinic acid with that of **6**. The mass spectrum of **5** showed characteristic peaks at *m/z* 222 and 191 due to cleavage of the C<sub>7</sub>/C<sub>8'</sub> and C<sub>7</sub>/C<sub>8</sub> bonds. From these results, the lignan part is fully represented by structure **5**. These facts lead to structure **2** for anthocerotonic acid. As (*R*)-rosmarinic acid is the major component in *Anthoceros punctatus*, it is likely that the new lignan **2** is directly derived from (*R*)-rosmarinic acid and *p*-coumaric acid in *A. punctatus*.



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#### References and notes

1. a) Takeda R.; Naoki H.; Iwashita T.; Mizukawa K.; Hirose Y.; Ishida T.; Inoue M.; *Bull. Chem. Soc. Jpn.*, **1983**, *56*, 1265. b) Takeda R.; Ohta Y.; Hirose Y.; *Bull. Chem. Soc. Jpn.*, **1983**, *56*, 1120. c) Takeda R.; Katoh K.; *J. Am. Chem. Soc.*, **1983**, *105*(12), 4056.
2. a) Kelley C. J.; Mahajan J. R.; Brooks L. C.; Neubert L. C.; Breneman W. R.; Carmack M.; *J. Org. Chem.*, **1975**, *40*, 1804. b) Kelley C. J.; Harruff R. C.; Carmack M.; *J. Org. Chem.*, **1976**, *41*, 449.
3. The physical constants of megacerotonic acid are as follows:  $C_{18}H_{14}O_7$  (SIMS,  $m/z$  343,  $[M+H]^+$ ),  $[\alpha]_D^{25} +233.0$  (c 1.66, 5% AcOH), IR (film)  $1735\text{cm}^{-1}$  (ester); UV (5% AcOH) :  $\lambda_{max}$  228 ( $\epsilon$  13,500), 289(sh.,  $\epsilon$  11,000) and 319 nm ( $\epsilon$ , 18,000);  $^1\text{H}$  NMR (300 MHz,  $\delta$ ppm,  $D_2O$ ) : 4.52 (1H, dd,  $J = 1.6, 3.0$  Hz, 7-H), 4.74 (1H, d,  $J = 3.0$  Hz, 8-H), 6.53 (1H, dd,  $J = 2.0, 8.2$  Hz, 6-H), 6.62 (2H, d,  $J = 8.6$  Hz, 3',5'-H), 6.70 (1H, d,  $J = 8.2$  Hz, 5-H), 6.82 (1H, d,  $J = 2.0$  Hz, 2-H), 7.14 (2H, d,  $J = 8.6$  Hz, 2',6'-H) and 7.58 (1H, d,  $J = 1.6$  Hz, 7'-H);  $^{13}\text{C}$  NMR (75.5MHz,  $\delta$ ppm,  $D_2O$ ) : CH- (50.6), HC-O (85.8), HC= (117.8), C= (161.0), two C=O (177.5 and 177.6) and twelve aromatic carbons(118.4 x 2, 119.3, 122.0, 123.9, 127.8, 134.8, 136.2 x 2, 144.0, 146.3 and 147.3).
4. Harada N.; Nakanishi K.; "Circular Dichroic Spectroscopy-Exciton Coupling in Organic Stereochemistry": University Science Books: Mill Valley, CA, 1983.
5. Anthocerotonic acid **2** exhibits the following constants:  $C_{27}H_{24}O_{11}$  (SIMS,  $m/z$  525  $[M+H]^+$ );  $[\alpha]_D^{25} +4.4$  (c=1.1, 5% AcOH); UV (5% AcOH) : 226 ( $\epsilon$  18,400) and 282 nm ( $\epsilon$  6,600);  $^1\text{H}$  NMR (300 MHz,  $\delta$ ppm,  $CD_3OD$ ): 2.88 (1H, dd,  $J = 9.7, 14.3$  Hz, 11- $H_A$ ), 3.07 (1H, t,  $J = 9.8$  Hz, 7- and 7'-H), 3.08 (1H, dd,  $J = 3.0, 14.3$  Hz, 11- $H_B$ ), 3.64 (1H, t,  $J = 9.8$  Hz, 8- $H^a$ ), 3.68 (1H, t,  $J = 9.8$  Hz, 8'- $H^a$ ), 5.13 (1H, dd,  $J = 3.0, 9.7$  Hz, 10-H), 6.45 (1H, dd,  $J = 1.8, 8.5$  Hz, 6- $H^b$ ), 6.60 (1H, dd,  $J = 1.8, 8.5$  Hz, 17- $H^b$ ), 6.64 (1H, d,  $J = 8.5$  Hz, 5- $H^c$ ), 6.71 (1H, d,  $J = 1.8$  Hz, 2- $H^d$ ), 6.75 (1H, d,  $J = 8.5$  Hz, 16- $H^c$ ), 6.77 (1H, d,  $J = 1.8$  Hz, 13- $H^d$ ), 6.78 (2H, d,  $J = 8.5$  Hz, 3'- and 5'-H), and 7.04 (2H, d,  $J = 8.5$  Hz, 2'- and 6'-H);  $^{13}\text{C}$  NMR (125.8 MHz,  $CD_3OD$ ,  $\delta$ ppm):  $CH_2$  (37.6), four -CH (43.5, 43.9, 50.1 and 50.4), -OCH (75.9), three C=O (177.0, 174.5 and 174.5), eighteen aromatic carbons (114.9, 116.4, 116.4, 116.6, 116.7, 117.4, 119.4, 122.1, 128.7, 128.7, 129.8, 133.3, 134.1, 144.6, 144.9, 145.8, 145.8 and 156.6).  
a, b, c, d Assignments may be interchangeable.