

## GRIMALDONE, A TRICYCLIC SESQUITERPENOID FROM *MANNIA FRAGRANS*. CRYSTAL STRUCTURE ANALYSIS.

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(Received 6 July 1987)

**Key Word Index**—*Mannia fragrans*, Aytoniaceae, Hepaticae, grimaldone, sesquiterpenoid, (−)-6S, 8R-cyclo-5R-cupar-9(15)-en-2-one

**Abstract**—The structure of grimaldone, a tricyclic sesquiterpenoid from the liverwort *Mannia fragrans*, has been established by crystal structure analysis. Its absolute configuration was assigned by comparison of its CD spectrum with that of (+)-S- $\alpha$ -cuparenone.

### INTRODUCTION

In Central Europe the genus *Mannia* (Marchantiales) comprises three species, *M. pilosa*, *M. triandra* and, the most common, *M. fragrans* (Balbis) Frye et Clark. *M. fragrans* grows on both siliceous and calcareous soils or sunny cliffs. It occurs in only a few locations in the G.D.R., Czechoslovakia and Poland but is abundant in Hungary, especially near Lake Balaton and north Budapest. As its name suggests *M. fragrans* emits a pleasant and intense odour when the thallus is wet and a sensitive nose can detect it at a distance of many metres. In contrast, in its dry state the plant has a very weak odour and is difficult to distinguish from the surrounding soil [1, 2].

From *M. fragrans* collected at Balatonfüred in Hungary Huneck and Schreiber [3] isolated the odoriferous principle,  $C_{15}H_{22}O$ , mp 91–92° and named it grimaldone after the old genus name *Grimaldia*. A year later Benesová [4] found (−)-R- $\alpha$ -cuparenone in *M. fragrans* collected in Southern Moravia but could not isolate grimaldone. Our supply (10 mg) of grimaldone disappeared from a vial with a plastic stopper in the course of a year because of the high vapour pressure of this sesquiterpenoid—it sublimes readily at room temperature. The next opportunity to collect larger amounts of the liverwort came to one of us (S H) during botanical expeditions to Mongolia in 1978 and 1983. Extraction of the plant material followed by chromatography afforded grimaldone.

### RESULTS AND DISCUSSION

Grimaldone (**1**) has resonances in its  $^1H$  and  $^{13}C$  NMR spectra for three tertiary methyls [ $\delta_H$  0.96 (*d*,  $J$  0.9 Hz), 1.01 and 1.05;  $\delta_C$  19.5, 21.1 and 21.2], a trisubstituted cyclopropane [ $\delta_H$  0.75 (*dd*,  $J$  = 4.0, 5.5 Hz), 1.62 (*dd*,  $J$  = 4.0, 8.4 Hz), 1.23 (*ddd*,  $J$  = 2.0, 5.5, 8.4 Hz);  $\delta_C$  16.1 (*t*, 27.2 (*d*), 37.1 (*s*))] and an exomethylene group [ $\delta_H$  4.66 and 4.85;  $\delta_C$  153.2 (*s*, 102.4 (*t*))]. The molecule also contains four methylenes [ $\delta_C$  27.9, 28.9, 29.2, 33.7], two quaternary carbons [ $\delta_C$  45.4, 53.4] and a cyclopentanone [ $\delta_C$  223.2,  $\nu_{max}$  ( $CCl_4$ ) 1742  $cm^{-1}$ ]. Thus grimaldone is

tricarbocyclic. The UV spectrum [ $\lambda_{max}^{MeOH}$  222 nm ( $\epsilon$  2630)] suggests that the cyclopropane and the exomethylene group are conjugated. Decoupling studies at 360 MHz enabled some of the proton connectivities to be established despite considerable overlap. Thus there is a methylene group [ $\delta_H$  2.35 (*ddd*,  $J$  = 2.7, 10.0, 19.7 Hz), 2.25 (*ddd*,  $J$  = 9.0, 9.8, 19.7 Hz)]  $\alpha$  to the carbonyl group with a neighbouring isolated methylene group [ $\delta_H$  1.78 (*ddd*,  $J$  9.8, 10.0, 12.6 Hz), 1.22 (*ddd*,  $J$  = 2.7, 9.0, 12.6 Hz)]. The cyclopropyl methine proton is coupled ( $J$  = 2.0 Hz) to one proton [ $\delta_H$  2.01 (*m*, clearly including *d*,  $J$  = 2.0 Hz)] of a further  $-CH_2CH_2-$  system [ $\delta_H$  2.18 (*m*), 2.09 (*m*, clearly including allylic couplings *t*,  $J$  = 2.4 Hz) and 1.75 (*m*, clearly including weak unresolved allylic couplings)]. Initially we interpreted this as a vicinal coupling and consequently we were unable to derive a rational structure for grimaldone. The problem was solved by a single crystal X-ray analysis which revealed the structure and relative stereochemistry of grimaldone as in **1**. An ORTEP diagram of the molecule is shown in Fig. 1. Both five-membered rings adopt twisted conformations and the bicyclohexane group is pseudoequatorial to the cyclopentanone. With hindsight it is clear that the coupling between the cyclopropyl methine and the methylene proton is a  $^4J$  interaction.

The absolute configuration of grimaldone as in **1** follows from comparison of its CD spectrum (Fig. 2) with that of (+)- $\alpha$ -cuparenone (**2**) which is assumed to have its *p*-tolyl group pseudoequatorial to the cyclopentanone ring. Such a twisted conformation would give rise [5] to a positive CD for **2** in agreement with the observed sign. Similarly the conformation of grimaldone **1** based on the X-ray crystal structure would give rise to a negative CD. Thus grimaldone is (−)-6S, 8R-cyclo-5R-cupar-9(15)-en-2-one, a derivative of *ent*-cuparane, and provides a further example of the capacity of liverworts to produce sesquiterpenoids enantiomerically related to those of higher plants [6]. Grimaldone is the first example of a sesquiterpenoid with this tricyclic carbon skeleton [7]. Recently Asakawa and his colleagues [8] have isolated the alcohol **3** from *Marchantia paleacea* Bertol. var *dipetra* (Mont) Hatt.

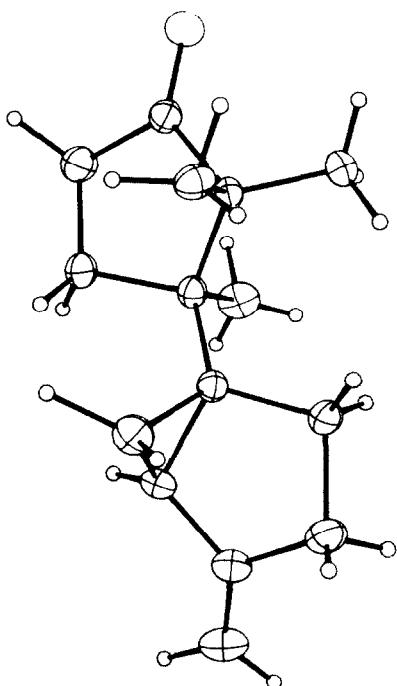
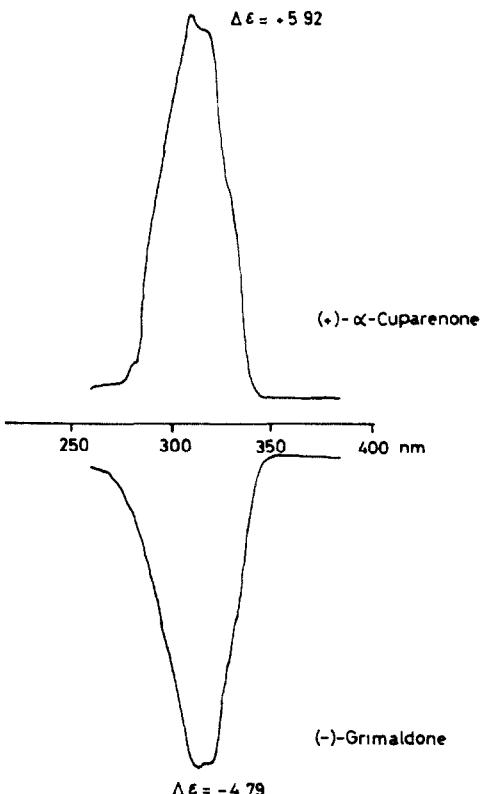
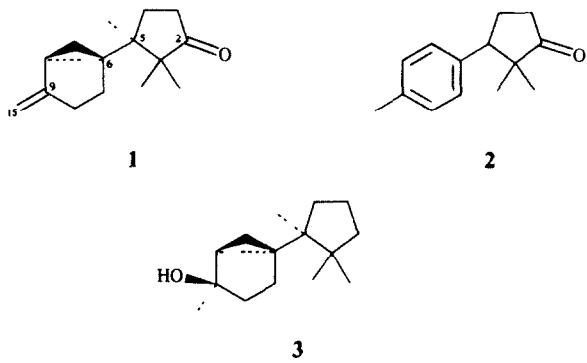


Fig. 1 ORTEP diagram of grimaldone

Fig. 2 CD spectra of grimaldone and (+)- $\alpha$ -cuparenone

## EXPERIMENTAL

*Isolation of grimaldone from M. fragrans* collected (i) near Ulan-Bator and (ii) in North Mongolia (i) The moist plant material ( $\sim 150$  g) (Mongolian Peoples Republic, Ulan-Bator, Zaisan Valley, 3 km S of Ulan-Bator, on soil in the mountain steppe [9], leg. S. Huneck, 26 July 1978, det. R. Grolle) was separated as well as possible from the adhering soil, minced in a beaker with an ultra-Turrax after the addition of a mixture of MeOH-Et<sub>2</sub>O (4:1, 150 ml) and the slurry filtered. The extract was evapd to dryness, the green waxy residue (1.4 g) redissolved in C<sub>6</sub>H<sub>6</sub>, the insoluble fraction removed and the soln chromatographed on silica gel (60 g, 5% H<sub>2</sub>O), after *n*-hexane (750 ml) *n*-hexane-Et<sub>2</sub>O [49:1 (1 l)] eluted a yellow oil which crystallized after the addition of a few drops of MeOH. The crystals were filtered, sublimed *in vacuo* at 120–140° and recrystallized from *n*-pentane to yield grimaldone (1) (0.022 g, 0.014%), C<sub>15</sub>H<sub>22</sub>O (218), in prisms of mp 91–92° and R<sub>f</sub> 0.58 [silica gel PF 254 + 366, C<sub>6</sub>H<sub>6</sub>-Et<sub>2</sub>O (9:1), 10% H<sub>2</sub>SO<sub>4</sub>, 150° → violet spot] MS, m/z 218 165 (M<sup>+</sup>, 11%, calc for C<sub>15</sub>H<sub>22</sub>O 218 167), 203 144 ([M

C<sub>7</sub>H<sub>10</sub> 94 078), 83 (C<sub>6</sub>H<sub>11</sub>, 100), IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup> 805, 866, 888, 915, 938, 958, 972, 984, 996, 1045, 1062, 1090, 1107, 1144, 1158, 1192, 1204, 1260, 1273, 1290, 1305, 1315, 1365, 1377, 1387, 1410, 1462, 1650, 1722, 2080, 3045, 3084,

$$[\alpha]^{24} \begin{array}{ccccccc} 589 & 578 & 546 & 436 & 406 & 366 \\ -49.6 & -55 & -79 & -206 & -312 & -643 \end{array} \text{ nm}$$

(CHCl<sub>3</sub>, c 0.635), ORD (3.74 mg/3 ml MeOH)  $[\phi]_{400} -600$ ,  $[\phi]_{312} -6200$ ,  $[\phi]_{296} \pm 0$ ,  $[\phi]_{274} + 7600$ ,  $[\phi]_{230} \pm 0$

(ii) The air-dried and ground liverwort (400 g) (Mongolian Peoples Republic, Chovsgol Aimak, Ulaan-uul Sum, on cliffs near the Tomyn-Brigade [10], leg. S. Huneck, 17 July 1983, det. R. Grolle, voucher specimens of both samples in the herbarium of S. H.) was extracted with Et<sub>2</sub>O, the solvent removed from the extract and the oily residue (3 g) chromatographed on silica gel (70 g, 5% H<sub>2</sub>O), fractions 1(*n*-hexane, 750 ml) colourless oil (0.2 g, not further investigated), 2(*n*-hexane, 1 l) orange wax (0.07 g, not further investigated), 3(*n*-hexane-Et<sub>2</sub>O, 24:1, 1.5 l) yellow oil (0.8 g), 4 (*n*-hexane-Et<sub>2</sub>O, 4:1, 500 ml) greenish oil

and crystals (0.6 g). Fraction 3 was further separated by prep. TLC on plates with silica gel PF 254 + 366 [20 × 20 × 0.1 cm, *n*-hexane-Et<sub>2</sub>O (4:1)]. After crystallization from *n*-pentane the band of *R*<sub>f</sub> 0.39 yielded grimaldone (33 mg, 0.083%) of mp 86–88°. Fraction 4 was rechromatographed and gave a mixture of steroids (0.02 g, 0.005%) of mp 128–130°, GLC-MS (steel column, 180 × 0.2 cm, SE 30, 250°) showed the presence of brassicasterol, campesterol, stigmasterol and sitosterol.

Crystal data C<sub>15</sub>H<sub>22</sub>O, *M* = 218.3, orthorhombic, *P*2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, *a* = 7.173(3), *b* = 8.833(1), *c* = 20.726(4) Å, *U* = 1313.0 Å<sup>3</sup>, *F*(000) = 480, *D*<sub>c</sub> = 1.10 g/cm<sup>3</sup>, *Z* = 4, MoK<sub>α</sub> radiation,  $\lambda$  = 0.71069 Å, 1055 independent observed reflections (*I* ≥ 3.0  $\sigma$ <sub>I</sub>) were collected on an Enraf-Nonius CAD-4 automatic diffractometer. The structure was solved by direct phasing techniques using MIT-HRIL [11]. Full matrix least-squares refinement of anisotropic parameters for non-hydrogen atoms and in the final two cycles the isotropic parameters of the hydrogen atom positions resulted in a final *R* of 0.047 and *wR* of 0.058 [*w* = 1/σ<sup>2</sup>(*F*<sub>0</sub>)] [The atomic coordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratories, Lensfield Road, Cambridge CB2 1EW.]

**Acknowledgements**—We are grateful to Prof G Posner, the Johns Hopkins University, Baltimore, for a sample of (+)-cuparenone, Dr I H Sadler and the SERC Highfield NMR Service, University of Edinburgh for 360 MHz NMR spectra

and Dr J Schmidt, IBP Halle, for the GLC-MS analysis of the steroids from *M. fragrans*.

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