# Novel Neurotrophic Isocuparane-type Sesquiterpene Dimers, Mastigophorenes A, B, C and D, Isolated from the Liverwort *Mastigophora diclados*

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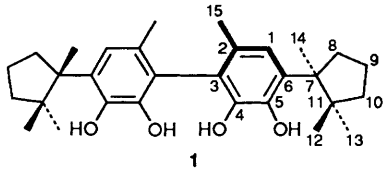
Four dimeric isocuparane-type sesquiterpenes, mastigophorenes A, B, C and D, isolated together with a new 3,4-dihydroxylated isocuparene from the liverwort *Mastigophora diclados*, have been assigned structures on the basis of detailed spectroscopic analyses. Among them, mastigophorenes A, B and D have been found to accelerate neuritic sprouting at $10^{-5}$–$10^{-7}$ mol $dm^{-3}$ in a neuritic cell culture of foetal rat cerebral hemisphere; their biosynthesis, initiated by phenolic oxidation of (−)-herbertenediol, is proposed.

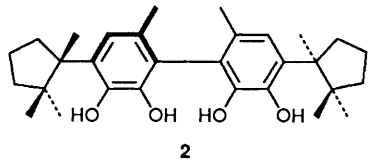
The liverworts (Hepaticae) contain a wide variety of terpenoids and lipophilic aromatic substances which constitute the characteristic oil bodies,[1] and we have very often encountered different types of biologically active compounds[2–4] (*e.g.*, antifeedant, plant-growth regulator, antimicrobial, cytotoxic, and tumour promoting) from the liverworts. A variety of metabolites in the liverworts continue to intrigue us because of their structural novelty, relative high concentration (*e.g.*, marchantin A[5] and plagiochiline A[6]) and interesting biological activity.

*Mastigophora diclados* (Brid.) Nees is a rather primitive liverwort and is commonly found in tropical Asiatic areas. GC-MS analysis of its ether extract implied the presence of cuparane and oxygenated isocuparane (herbertane[7])-type sesquiterpenes.[8] The original study of *M. diclados* reported the isolation of the several known cuparane and isocuparane-type sesquiterpenes.[9] Our independent chemical study on the ether extract of *M. diclados* collected in Borneo resulted in the isolation of four isocuparane-type sesquiterpenes, compounds **1**, **2**, **6**[10] and **7**,[10] named mastigophorene A, B, C and D, respectively, which possess a unique dimeric structure of isocuparene, as well as of a new isocuparene-3,4-diol **3** and known (−)-herbertenediol **4**[11] and β-herbertenol **5**.[12] Among them, mastigophorenes A, B and D have been found to exhibit interesting neurotrophic activity.[13,14] In this paper we report the structures of these new compounds and their neurotrophic properties, and also a biosynthetic pathway to the four dimers from (−)-herbertenediol **4** will be proposed.

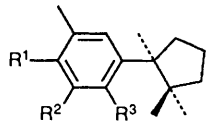
Mastigophorenes A **1** and B **2** had the same molecular formula $C_{30}H_{42}O_4$, confirmed by their high-resolution mass spectra, and revealed absorption bands attributable to a hydroxy group (3550 $cm^{-1}$) and a benzene ring in their IR and UV spectra. The $^1H$ and $^{13}C$ NMR spectra (Table 1) for compounds **1** and **2** were almost identical and indicated the presence of three tertiary methyl and one aromatic methyl groups, and a pentasubstituted benzene ring since there was only one aromatic proton signal, observed at $\delta_H$ 6.86 (s) and 6.85 (s) for compounds **1** and **2**, respectively. It was also noted that the $^1H$ and $^{13}C$ NMR data were closely related to those of herbertenediol **4**, which is as a main constituent in the title plant, except for the lack of the one of the *meta*-coupled aromatic proton signals shown for compound **4**, and the replacement of the aromatic methine carbon signal at $\delta_C$ 113.4 in herbertenediol **4** with the aromatic quaternary carbon signal at $\delta_C$ 117.09 and 117.04 for compound **1** and **2**, respectively. The spectral evidence mentioned above is other-



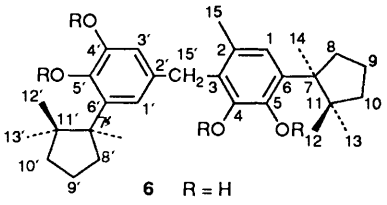

**1**



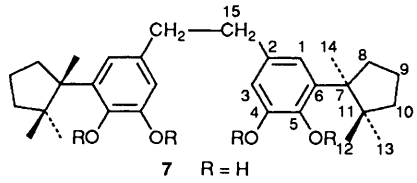

**2**




**3** $R^1 = R^2 = OH$, $R^3 = H$
**3a** $R^1 = R^2 = OAc$, $R^3 = H$
**4** $R^1 = H$, $R^2 = R^3 = OH$
**5** $R^1 = OH$, $R^2 = R^3 = H$




**6** R = H
**6a** R = Ac




**7** R = H
**7a** R = Ac

**Table 1** $^{13}C^{a}$ and $^{1}H$ NMR data (in $CDCl_3$) for compounds **1**–**4**

| | **1** | | **2** | | **3** | | **4** (ref. 9) | |
|---|---|---|---|---|---|---|---|---|
| Carbon | C | H | C | H | C | H | C | H |
| 1 | 122.71 | 6.86s | 122.66 | 6.85s | 121.56 | 6.67d (*J* 2.2) | 121.8 | 6.67d (*J* 2.2) |
| 2 | 126.70 | | 126.71 | | 123.04 | | 128.0 | |
| 3 | 117.09 | | 117.04 | | 139.61 | | 113.4 | 6.54d (*J* 2.2) |
| 4 | 140.59 | | 140.66 | | 142.22 | | 143.3 | |
| 5 | 141.64 | | 141.64 | | 112.22 | 6.74d (*J* 2.2) | 141.1 | |
| 6 | 133.88 | | 133.85 | | 140.02 | | 133.4 | |
| 7 | 51.36 | | 51.37 | | 50.03 | | 51.1 | |
| 8 | 38.93 | 2.68m, 1.75m | 39.18 | 2.74m, 1.75m | 36.91 | 2.90m, 1.78m | 40.9 | |
| 9 | 20.48 | 1.69m | 20.48 | 1.69m | 19.61 | 1.62m | 21.1 | |
| 10 | 41.22 | 1.69m | 41.25 | 1.69m | 39.75 | 1.62m | 40.9 | |
| 11 | 44.98 | | 44.98 | | 44.14 | | 44.8 | |
| 12 | 25.55 | 1.21s | 25.82 | 1.21s | 24.26 | 1.07s | 25.4 | 1.17s |
| 13 | 27.23 | 0.80s | 27.15 | 0.79s | 26.56 | 0.56s | 27.1 | 0.75s |
| 14 | 22.87 | 1.46s | 22.60 | 1.47s | 24.56 | 1.20s | 22.8 | 1.40s |
| 15 | 19.21 | 1.94s | 19.22 | 1.93s | 15.89 | 2.24s | 20.4 | 2.21s |
| OH | | 4.77s, 5.59s | | 4.77s, 5.58s | | | | |

[a] Assignments were made by $^{13}C$–$^{1}H$ and long-range $^{13}C$–$^{1}H$ COSY experiments.

**Table 2** $^{13}C$–$^{1}H$ correlation in the long-range $^{13}C$–$^{1}H$ COSY experiments for compounds **1**, **2**, **6** and **7a**

| C | H in **1** and **2** | H in **6** | H in **7a** |
|---|---|---|---|
| 1 | 15-$H_3$ | 15-$H_3$ | 15-$H_3$, 3-H |
| 1′ | | 15′-$H_2$, 3′-H | |
| 2 | 15-$H_3$ | 15-$H_3$, 15′-$H_2$ | 15-$H_3$ |
| 2′ | | 15′-$H_2$ | |
| 3 | 1-H, 15-$H_3$ | 1-H, 15-$H_3$, 15′-$H_2$ | 1-H, 15-$H_3$ |
| 3′ | | 1′-H, 15′-$H_2$ | |
| 4 | 1-H | 15′-$H_2$ | |
| 5 | 1-H | 1-$H_2$ | 1-, 3-H |
| 5′ | | 1′-, 3′-H | |
| 6 | 14-$H_3$ | 14-$H_3$ | 14-$H_3$ |
| 6′ | | 14′-$H_3$ | |
| 7 | 1-H, 12-, 13-, 14-$H_3$ | 1-H, 12′-, 13′-$H_3$ | 1-H, 12-H, 13-$H_3$ |
| 7′ | | 1′-H, 12′-, 13′-$H_3$ | |
| 8 | 14-$H_3$ | 14-$H_3$ | 14-$H_3$ |
| 8′ | | 14′-$H_3$ | |
| 10 | 12-, 13-$H_3$ | 12-, 13-$H_3$ | 12-, 13-$H_3$ |
| 10′ | | 12′-, 13′-$H_3$ | |
| 11 | 12-, 13-, 14-$H_3$ | 12-, 13-, 14-$H_3$ | 12-, 13-, 14-$H_3$ |
| 11′ | | 12′-, 13′-, 14′-$H_3$ | |
| 12 | 13-$H_3$ | 13-$H_3$ | 13-$H_3$ |
| 12′ | | 13′-$H_3$ | |
| 13 | 12-$H_3$ | 12-$H_3$ | 12-$H_3$ |
| 13′ | | 12′-$H_3$ | |
| 15 | 1-H | 1-H | 1-, 3-H |
| 15′ | | 1′-, 3′-H | |

wise compatible with symmetrical dimers of herbertenediol **4**, presumably linked through an aryl–aryl bond at C-1 or C-3 in herbertenediol **4**, and thus the spectral unequivalence between compounds **1** and **2** could be rationalized as being due to diastereomeric environment caused by atropisomers with respect to the biphenyl bond. The aryl–aryl bonds in compounds **1** and **2** should be formed between the C-3 positions on the two molecules of herbertenediol **4**, since the chemical-shift values for the quaternary carbons involving the biphenyl bond were observed at relatively high field ($\delta_C \sim 117$). The sole aromatic proton at $\delta_H$ 6.86 or 6.85 showed an NOE interaction with the 14-$H_3$ methyl signal. In addition, these tentative structures for compounds **1** and **2** were supported by the long-range $^{13}C$–$^{1}H$ COSY experiments summarized in Table 2, in which the 15-$H_3$ methyl proton signal correlated to the C-1, C-2 and C-3 carbon signals, and the 1-H proton signal showed correlations with the C-3 and C-5 carbons. These spectral data led us to propose the structures **1** and **2** which must be atropisomers at the biphenyl axis linked at the C-3 positions on the two molecules of herbertenediol **4**. In order to clarify the absolute configurations at the aryl–aryl axis of compounds **1** and **2**, the CD exciton chirality rule was applied.[15] The CD spectrum of compound **1** showed the first (positive) Cotton effect at 222 nm and the second (negative) Cotton effect at 202 nm, indicating an (*S*)-configuration at the biaryl axis, whereas compound **2** had the (*R*)-configuration due to the first (negative) and second (positive) Cotton effects at 215 and 202 nm, respectively. Accordingly, the structures of mastigophorenes **A 1** and **B 2** were assigned to (*S*)-3,3′-biherbertenediol and (*R*)-3,3′-biherbertenediol, respectively.

The high-resolution EI mass spectrum of compound **3**, m.p. 150–151 °C, gave the molecular formula $C_{15}H_{22}O_2$ [*m/z* 234.1630 (calc. 234.1629)], equivalent to that of herbertenediol **4**, and base fragments at *m/z* 164 and 152 which also occurred in the mass spectrum of compound **4**. The usual acetylation of compound **3** yielded the diacetate **3a** ($\delta_H$ 2.27 and 2.29), indicating the presence of two phenolic hydroxy groups. These physical data suggest that compound **3** belongs to the isocuparene-type sesquiterpenes. The $^{1}H$ NMR spectrum (Table 1) contained the same spin systems as in compound **4**; *i.e.* three tertiary methyl groups, one aromatic methyl group and a 1,2,3,5-tetrasubstituted benzene ring, whereas the $^{13}C$ NMR data (Table 1) for the cyclopentane part were almost identical with those of herbertenediol **4**, but those for the benzene ring were not consistent with those of compound **4**. This implied that the benzene ring of compound **3** had an arrangement of substitutents (2 × OH, Me) different from that in compound **4**. Difference NOE data were informative of solving this ambiguity. Upon irradiation of the 15-$H_3$ methyl signal at $\delta_H$ 2.24 an NOE was observed for the 1-H at $\delta_H$ 6.67, whereas both 1-H and 5-H ($\delta_H$ 6.74) showed distinct NOE enhancements upon irradiation of the 14-$H_3$ methyl signal at $\delta_H$ 1.20. This result unambiguously showed that the aromatic methyl and two hydroxy groups were in turn arranged at the C-2, C-3 and C-4 positions, respectively. Thus, the structure of compound **3** was determined as isocuparene-3,4-diol.

Mastigophorene C **6** had the same molecular formula $C_{30}H_{42}O_4$ as compounds **1** and **2**, obtained from its high-resolution mass spectrum [*m/z* 466.3083 (calc. 466.3083)] and exhibited hydroxy and aromatic absorption bands at 3530 $cm^{-1}$, and 216

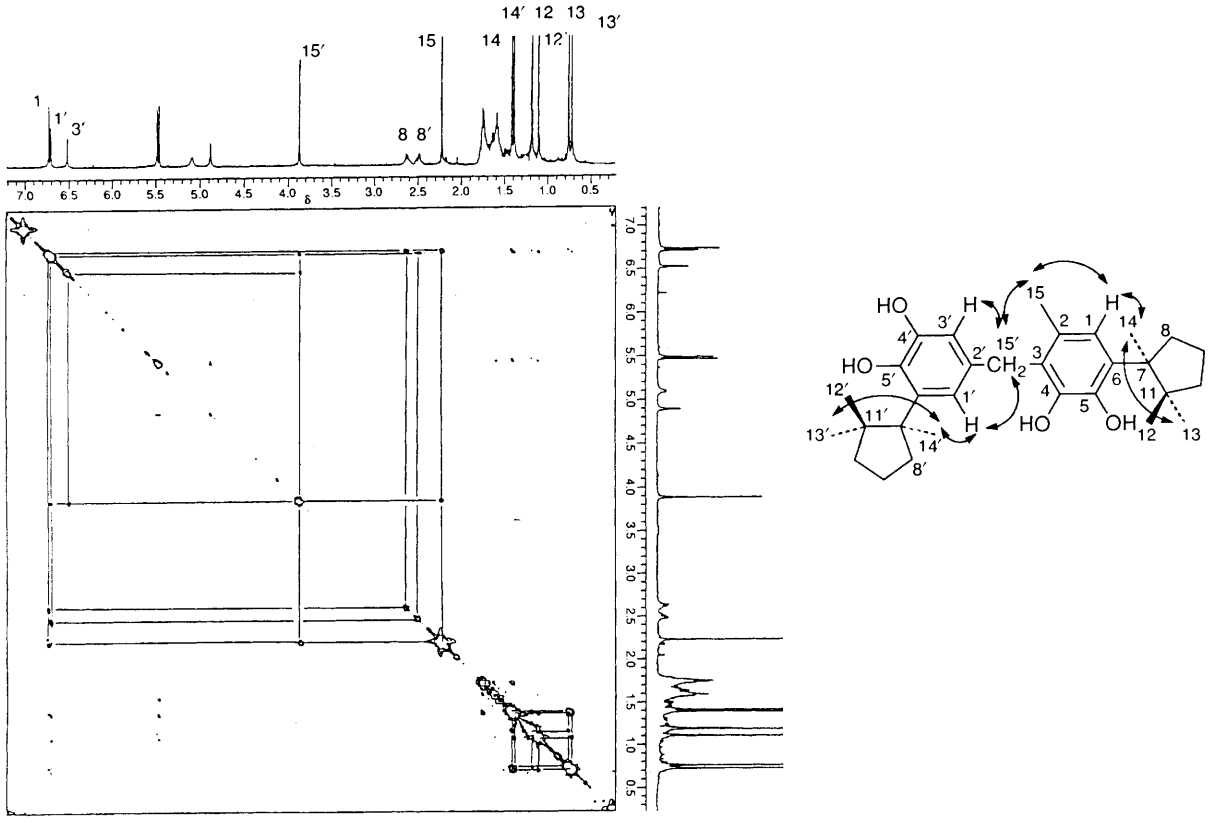



**Fig. 1** NOESY spectrum (PD 4 s; mixing time 0.75 s) of mastigophorene C **6**

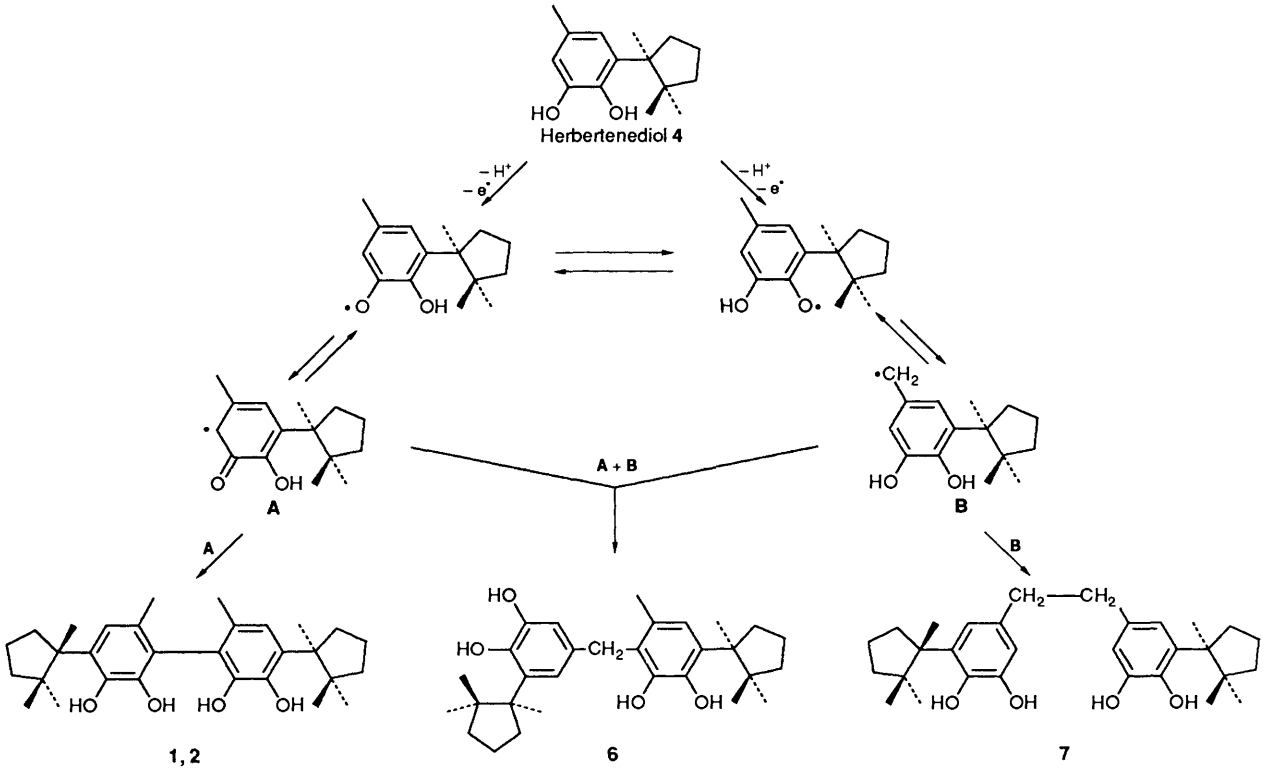
and 280 nm, in its IR and UV spectra, respectively. The $^1H$ and $^{13}C$ NMR data (Table 3) of compound **6** indicated the presence of a set of three tertiary methyl groups and one benzylic methylene group at $\delta_H$ 3.82 (2 H, s) and $\delta_C$ 32.16, and a 1,3,4,5-tetrasubstituted benzene ring [$\delta_H$ 6.51 (d, $J$ 1.8 Hz) and 6.70 (d, $J$ 1.8 Hz), as well as of a pentasubstituted benzene ring [$\delta_H$ 6.76 (s)] appended with a methyl group ($\delta_H$ 2.32). Acetylation of compound **6** with $Ac_2O$–pyridine yielded a fully acetylated derivative **6a**, the $^1H$ NMR of which revealed the presence of four aromatic acetyl signals ($\delta_H$ 2.17, 2.23, 2.25 and 2.27), disclosing that compound **6** has four phenolic hydroxy groups. These spectral features suggested that compound **6** comprised another asymmetric dimeric structure derived from (−)-herbertenediol **4**. In the long-range $^{13}C$–$^1H$ COSY spectrum of compound **6**, summarized in Table 2, the aromatic methyl proton signal (15-$H_3$) at $\delta_H$ 2.32 was correlated to the carbon signals at $\delta_C$ 122.33 (C-1), 126.73 (C-2) and 123.16 (C-3), and also the benzylic proton signal (15′-H) showed clear cross-peaks with the aromatic carbon signals at $\delta_C$ 121.16 (C-1′), 129.37 (C-2′), 112.40 (C-3′), 126.73 (C-2) and 123.16 (C-3). These results indicated that the array of the substituents on the benzene ring not only corresponded to that of (−)-herbertenediol **4**, but also that the C-15 methyl group of one molecule of compound **6** should be linked to the C-3 position on the benzene nucleus of another molecule of compound **6**. In addition, the 2-D NOESY spectrum of **6**, as shown in Fig. 1, substantiated the foregoing assignment for compound **6**. Therefore the structure of mastigophorene C must be represented as structure **6**.

Another dimer, mastigophorene D **7**, m.p. 201–203 °C, had the same molecular formula $C_{30}H_{42}O_4$ as compound **6**, based on its high-resolution mass spectrum and elemental analysis, and contained phenolic hydroxy groups and a benzene nucleus, supported by its IR and UV spectra, and the usual acetylation features (see Experimental section). The $^1H$ and $^{13}C$ NMR spectra (Table 3) for compound **7**, however, were again very similar to those of herbertenediol **4**, the major difference being the observation of a new signal ($\delta_H$ 2.80, $\delta_C$ 37.69) due to a benzylic methylene group instead of the aromatic methyl signal found for compound **4**. This spectral difference reflects the fact that compound **7** has a symmetrical dimeric structure through the single bond formed between the C-15 aromatic methyl groups in herbertenediol **4**. In addition, the EI-MS spectrum of compound **7** displayed a base fragment ion peak at $m/z$ 233 corresponding to the herbertenediol benzyl cation, thereby supporting the dimeric structure which was assembled from two molecules of herbertenediol **4** at the C-15 position. This was substantiated by the long-range $^{13}C$–$^1H$ COSY (Table 2) and 2D NOESY experiments for the acetate derivative **7a** as follows: the 15-$H_2$ benzylic proton resonance at $\delta_H$ 2.90 correlated to the aromatic C-1, C-2 and C-3 carbon signals at $\delta_C$ 126.74, 138.16 and 120.72, respectively, whereas there were the NOE interactions observed between the 15-$H_2$ and 1- and 3-H ($\delta_H$ 7.09 and 6.91). Hence these results fully corroborated the structure **7** for mastigophorene D.

Although various *ent*-cuparane and isocuparane (herbertane)-type sesquiterpenes occur in the liverworts,[16] and are regarded as having significant biochemical characteristics,[8,9,12] the dimeric compounds such as **1**, **2**, **6**[10] and **7**[10] have not previously been recorded. Since these dimers could be significant in relation to the biogenesis initiated by phenolic oxidation from (−)-herbertenediol **4** which is a co-metabolite in the title plant, it was of interest to attempt to propose a biosynthetic relationship between these isocuparene dimers and isocuparene. They are presumably biosynthesized *via* phenoxy radicals produced by one-electron oxidation from (−)-herbertenediol **4**. The formed phenoxy radicals subsequently interconverted into a radical **A** or an unstable benzyl radical **B** which might evolve a quinonemethide with one more oxidation or the loss of $H^{\cdot}$.[17] Homocoupling between the two radicals **A** should lead to mastigophorenes A **1** and B **2** followed by aromatization, whereas mastigophorene D **7** is most likely to be

**Table 3** $^{13}C$ and $^{1}H$ NMR data (in $CDCl_3$) for compounds **6** and **7**

| | 6 | | 7 | |
|---|---|---|---|---|
| Carbon | C | H | C | H |
| 1, 1′ | 122.33, 121.16 | 6.76s, 6.70d (*J* 1.8) | 121.93 | 6.64d (*J* 2.0) |
| 2, 2′ | 126.73, 129.37 | | 132.3 | |
| 3, 3′ | 123.16, 112.40 | 6.51d (*J* 1.8) | 112.93 | 6.49d (*J* 2.0) |
| 4, 4′ | 142.20, 143.85 | | 143.26 | |
| 5, 5′ | 141.85, 141.95 | | 141.39 | |
| 6, 6′ | 131.12, 133.91 | | 133.28 | |
| 7, 7′ | 51.21, 50.97 | | 51.18 | |
| 8, 8′ | 39.09, 39.18 | 2.61m, 2.49m; 1.75m, 1.75m | 39.23 | 2.58m, 1.74m |
| 9, 9′ | 20.31, 20.25 | 1.75m, 1.75m | 20.33 | 1.74m |
| 10, 10′ | 40.91, 40.71 | 1.62m, 1.62m | 40.91 | 1.58m |
| 11, 11′ | 44.84, 45.05 | | 44.91 | |
| 12, 12′ | 25.41, 25.20 | 1.17s, 1.09s | 25.39 | 1.16s |
| 13, 13′ | 26.89, 26.43 | 0.76s, 0.72s | 26.79 | 0.73s |
| 14, 14′ | 22.86, 22.95 | 1.41s, 1.38s | 22.93 | 1.39s |
| 15, 15′ | 19.78, 32.16 | 2.32s, 3.82s | 37.69 | 2.80s |
| OH | | 4.85s, 5.14s 5.46s, 5.48s | | 4.95s 5.37s |




**Scheme 1** Plausible biosynthetic route to dimeric isocuparenes **1**, **2**, **6** and **7** based on one-electron oxidative coupling from (−)-herbertenediol **4**

derived from the direct coupling of the two benzyl radicals **B**. Another hetero coupling between the radicals **A** and **B** should give mastigophorene **C** (Scheme 1). The intermediary of the quinonemethide involving these radical dimerizations could be excluded since the formation of a dihydrostilbene is not explainable by this means. From a biosynthetic point of view we therefore suggest that the four dimeric isocuparenes isolated at the present study might originate from (−)-herbertenediol **4**.

Finally, it is worthy of note that mastigophorenes A, B and D exhibited interesting neurotrophic properties at $10^{-5}$–$10^{-7}$ mol $dm^{-3}$, which could greatly accelerate neuritic sprouting and network formation in the primary neuritic cell culture derived from the foetal rat hemisphere,[18] but mastigophorene C and the monomeric isocuparenes **3**, **4** and **5** suppressed neuritic differentiation.

## Experimental

M.p.s were obtained on a Yanagimoto hot-stage apparatus and are corrected. IR spectra were recorded in $CHCl_3$ solution on a Hitachi IR 260-10 spectrometer. Mass spectra were taken on a JEOL JMS-HX-100 spectrometer. NMR spectra were recorded on a JEOL GX-400 spectrometer operating at 400 MHz for $^{1}H$ and 100 MHz for $^{13}C$ nuclei. NOE and 2-dimensional experiments were performed on the same apparatus. Chemical shifts are reported in ppm relative to tetramethylsilane as internal standard. *J*-Values are given in Hz. Optical rotations were taken with a JASCO DIP-181 spectrometer. CD spectra were recorded on a JASCO J-500C spectropolarimeter. Wako silica gel C-300 (200–300 mesh) was used for column chromatography.

*Extraction and Isolation.*—The dried whole plant (220 g) of

*Mastigophora diclados* (Brid.) was immersed in diethyl ether at room temperature for 1 month and the obtained extract (6.0 g) was chromatographed on silica gel (160 g) with stepwise gradient elution [hexane–EtOAc (4:1) ⟶ (1:1)] to give fractions (fr.) 1–8 (each 100 $cm^3$). Fr. 1 (1.5 g) was chromatographed on Sephadex LH-20 (200 $cm^3$) with the solvent system MeOH–$CHCl_3$ (7:3) and then on silica gel (60 g) with hexane–$CH_2Cl_2$ (1:1) as eluent to give mastigophorene A **1** (36.2 mg), mastigophorene B **2** (61 mg) and herbertenediol **4** (300 mg).

Combined fr.s 2–4 (2.2 g) were chromatographed on Sephadex LH-20 (300 $cm^3$) and eluted with MeOH–$CHCl_3$ (7:3) followed by rechromatography on a silica gel column [hexane–EtOAc (4:1)] to afford β-isocuparenenol **5** (1.3 g), isocuparene-1,3-diol **3** (20 mg) and mastigophorene C **6** (30 mg).

Combined fr.s 6–8 (1.35 g) were rechromatographed on Sephadex LH-20 (500 $cm^3$) [MeOH–$CHCl_3$ (7:3)] and then on silica gel (20 g) [hexane–EtOAc (4:1)] to give mastigophorene D **7** (70 mg).

*Mastigophorene A* **1**. Needles, m.p. 258–261 °C (from hexane) (Found: C, 75.2; H, 9.2. $C_{30}H_{42}O_4 \cdot \frac{2}{3}H_2O$ requires C, 75.27; H, 9.12%); $[\alpha]_D^{19}$ −65.3 (*c* 0.4, $CHCl_3$); CD (EtOH) Δε (222 nm) +4.4, Δε (202 nm) −24.1; $\lambda_{max}$(EtOH)/nm 213 (ε 31 000) and 287 (3700); $\nu_{max}/cm^{-1}$ 3550 (OH); *m/z* (%) 466.3084 (100, $M^+$. $C_{30}H_{42}O_4$ requires M, 466.3083), 423 (10), 396 (15) and 384 (33). $^1H$ and $^{13}C$ NMR data: see Table 1.

*Mastigophorene B* **2**. Needles, m.p. 210–211 °C (from hexane) (Found: C, 72.25; H, 9.15. $C_{30}H_{42}O_4 \cdot \frac{2}{3}H_2O$ requires C, 75.27; H, 9.12%); $[\alpha]_D^{19}$ −39.1 (*c* 0.35, $CHCl_3$); CD (EtOH) Δε (215 nm) −15.5, Δε (202 nm) +24.6; $\lambda_{max}$(EtOH)/nm 218 (ε 34 000) and 287 (5000); $\nu_{max}/cm^{-1}$ 3550 (OH); *m/z* 466.3084 (100, $M^+$. $C_{30}H_{42}O_4$ requires M, 466.3083), 423 (10), 396 (15) and 384 (50). $^1H$ and $^{13}C$ NMR data: see Table 1.

*Isocuparene-3,4-diol* **3**. Needles, m.p. 150–151 °C (from hexane), $[\alpha]_D^{20}$ −73.6 (*c* 0.35, $CHCl_3$); $\lambda_{max}$(EtOH)/nm 219 (ε 19 200) and 285 (2300); $\nu_{max}/cm^{-1}$ 3550 (OH) and 1605 (benzene); *m/z* (%) 234.1630 (76, $M^+$. $C_{15}H_{22}O_2$ requires M, 234.1629), 164 (100) and 152 (87). $^1H$ and $^{13}C$ NMR data: see Table 1.

*Acetylation of* 3,4-*isocuparene*-3,4-*diol* **3**. Isocuparene-3,4-diol **3** (3 mg) was dissolved in a mixture of $Ac_2O$ (0.5 $cm^3$) and pyridine (1 $cm^3$). After 1 day at room temperature the solution was evaporated under reduced pressure and the residue was purified by silica gel chromatography to yield the diacetate **3a** as needles (3 mg), m.p. 65.5–66.5 °C (from MeOH); $\nu_{max}/cm^{-1}$ 1780 (CO), 1595 and 1490 (benzene); *m/z* (%) 318 (11, $M^+$), 276 (71, M − 42), 234 [100, M − (2 × 42)], 206 (27) and 152 (66); $\delta_H(CDCl_3)$ 0.59 (3 H, s, 13-$H_3$), 1.04 (3 H, s, 12-$H_3$), 1.24 (3 H, s, 14-$H_3$), 2.18 (3 H, s, 15-$H_3$), 2.27 (3 H, s, COMe), 2.29 (3 H, s, COMe), 6.77 (1 H, d, *J* 2.2, 1-H) and 7.05 (1 H, d, *J* 2.2, 5-H).

*Mastigophorene C* **6**. Oil, $[\alpha]_D^{20}$ −46.7 (*c* 0.4, $CHCl_3$); $\lambda_{max}$(EtOH)/nm 216 (ε 16 300), 280 (3000); $\nu_{max}/cm^{-1}$ 3530 (OH); *m/z* (%) 466.3082 (100, $M^+$. $C_{30}H_{42}O_4$ requires M, 466.3083), 348 (22, M − 82) and 247 (61); $^1H$ and $^{13}C$ NMR data: see Table 3.

*Acetylation of mastigophorene C* **6**. Mastigophorene C **6** (5 mg) was dissolved in a mixture of $Ac_2O$ (1 $cm^3$) and pyridine (2 $cm^3$), and then the solution was stored at room temperature for 1 day before being poured into ice–water and then extracted with $Et_2O$ (× 3). The usual work-up afforded the tetraacetate **6a** (5.5 mg) as an oil, $\lambda_{max}$(EtOH)/nm 249 (ε 22 500) and 268 (1100); $\nu_{max}/cm^{-1}$ 1767 (CO); *m/z* (%) 634 (26, $M^+$), 592 (32, M − 42), 574 (19, M − 60), 550 [100, M − (2 × 42)], 532 (27, M −42 − 60), 508 [20, M − (3 × 42)], 490 (30), 466 (10) and 273 (23); $\delta_H(CDCl_3)$ 0.63 (0.63 (3 H, s), 0.75 (3 H, s), 0.95 (3 H, s), 1.13 (3 H, s), 1.21 (3 H, s), 1.27 (3 H, s), 2.13 (3 H, s), 2.17, 2.23, 2.25 and 2.27 (each 3 H, s, COMe), 3.80 (1 H, d, *J* 16, 5′-H), 3.91 (1 H, d, *J* 16, 15′-H), 6.83 (1 H, d, *J* 2.0, 3′-H), 6.91 (1 H, d, *J* 2.0, 1′-H) and 7.15 (1 H, br s, 1-H).

*Mastigophorene D* **7**. Prisms, m.p. 201–203 °C (from hexane) (Found: C, 76.2; H, 9.1. $C_{30}H_{42}O_4 \cdot \frac{1}{3}H_2O$ requires C, 76.23; H, 9.11%); $[\alpha]_D^{23}$ −46.1 (*c* 0.5, $CHCl_3$); $\lambda_{max}$(EtOH)/nm 217 (ε 31 000) and 288 (8900); $\nu_{max}/cm^{-1}$ 3550 (OH) and 1600 (benzene); *m/z* (%) 466.3083 (76, $M^+$. $C_{30}H_{42}O_4$ requires M, 466.3083), 382 (12), 233 (100), 177 (36), 163 (57) and 151 (51). $^1H$ and $^{13}C$ NMR data: see Table 3.

*Acetylation of mastigophorene D* **7**. Mastigophorene D **7** (2 mg) was dissolved in a mixture of $Ac_2O$ (0.5 $cm^3$) and pyridine (1 $cm^3$) and the solution was stored at room temperature for 1 day before being poured into ice–water and then extracted with diethyl ether. Usual work-up gave the tetraacetate **7a** as a powder; $\nu_{max}/cm^{-1}$ 1760 (COMe) and 1603 and 1580 (benzene); *m/z* (%) 634 (16, $M^+$), 592 (27, M − 42), 550 [100, M − (2 × 42)], 532 (11), 508 (8), 490 (13), 233 (31), 177 (26), 163 (31) and 149 (51); $\delta_H(CDCl_3)$ 0.72 (3 H, s, 13-$H_3$), 1.12 (3 H, s, 12-$H_3$), 1.26 (3 H, s, 14-$H_3$), 2.24 and 2.28 (each 3 H, COMe), 2.90 (2 H, s, 15-$H_2$), 6.91 (1 H, d, *J* 2.0, 3-H) and 7.09 (1 H, d, *J* 2.0, 1-H); $\delta_C(CDCl_3)$ 20.11 (C-9), 20.79 (COMe), 21.04 (COMe), 23.28 (C-14), 24.94 (C-12), 26.24 (C-13), 37.03 (C-15), 38.64 (C-8), 40.15 (C-10), 45.29 (C-11), 51.35 (C-7), 120.72 (C-3), 126.74 (C-1), 138.16 (C-2), 139.40 (C-5), 140.34 (C-6), 142.62 (C-4), 168.20 (CO) and 168.47 (CO).

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