

Highlights in phytochemistry of hepaticae-biologically active terpenoids and aromatic compounds

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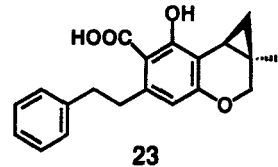
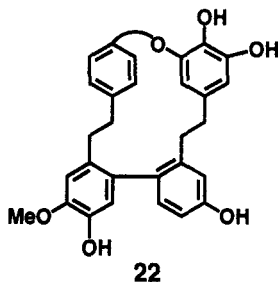
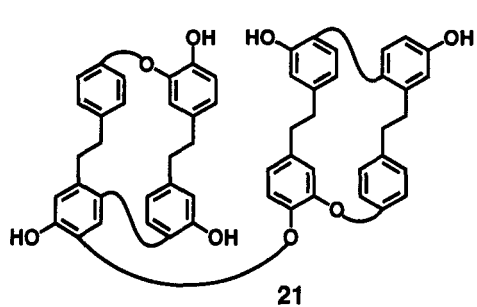
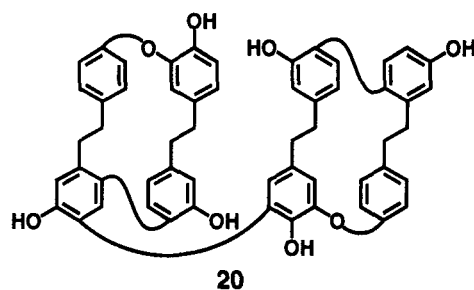
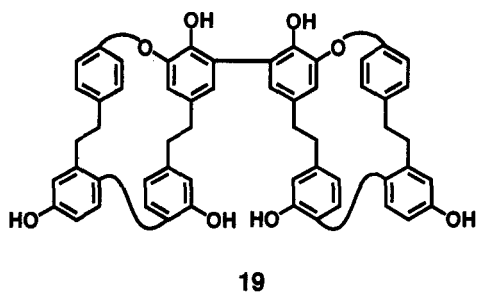
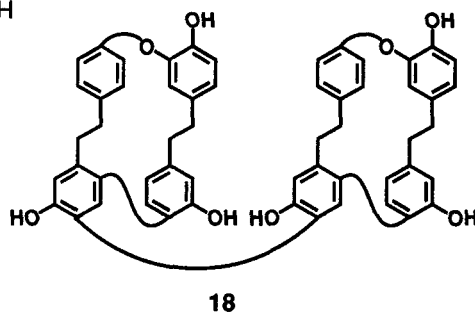
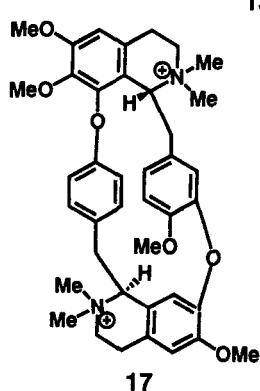
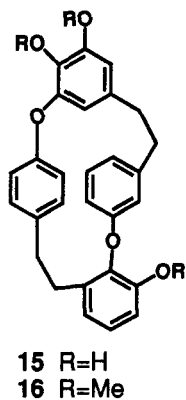
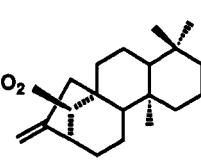
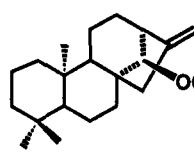
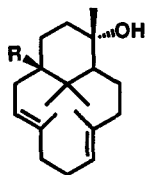
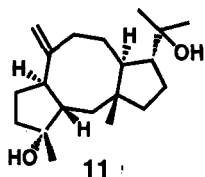
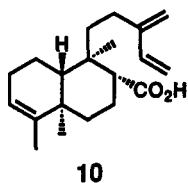
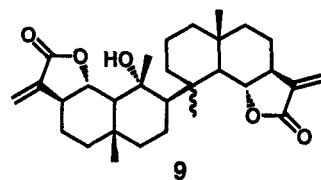
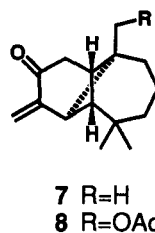
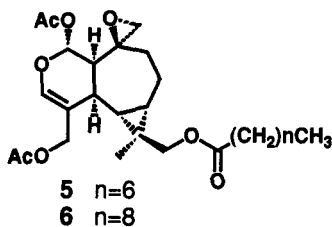
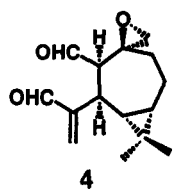
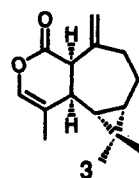
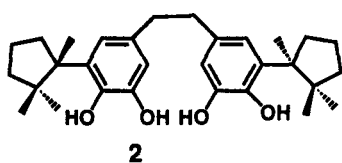
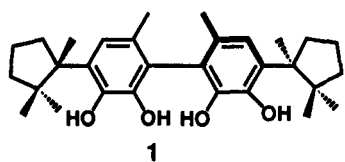
Abstract: The isolation and chemical structures of new terpenoids and aromatic compounds from several liverworts (Hepaticae) and their biological activities (neuritic sprouting, cytotoxic, antitumor activity, muscle relaxing, antimicrobial and antifungal activity, 5-lipoxygenase and calmodulin inhibitory activity) will be discussed.

There are about 6000 species of Hepaticae (liverworts) in the world. Some liverworts show characteristically fragrant odours and intensely hot and bitter taste as well as diuretic and anticancer activity. Generally, liverworts are not damaged by bacteria, fungi, insect larvae, snails, slugs and other small animals. Furthermore, some liverworts cause intense allergic contact dermatitis and allelopathy. A number of liverworts have been used as medicinal plants to treat burns, bruises, external wound. We have been interested in these biologically active substances found in Hepaticae and have studied about 750 species with respect to their chemistry, pharmacology, and application as sources of cosmetics, and medicinal or agricultural drugs.¹⁻⁴⁾ It has been demonstrated that most of the liverworts contain mainly sesquiterpenoids and lipophilic aromatic compounds. The biological activities of liverworts are due to these substances. In this paper, more recently isolated biologically active compounds and their biological activities will be summarized.

Terpenoids. Mastigophorenes A (1) and D (2) from *Mastigophora diclados* showed neurotrophic properties at 10^{-5} to 10^{-7} M, causing greatly accelerated neuritic sprouting and network formation in the primary neuritic cell culture derived from the fetal rat hemisphere. Plagiochilide (3) and plagiochilal B (4) from *Plagiochila fruticosa* show not only acceleration of neuritic sprouting but also enhancement of choline acetyl transferase activity at 10^{-5} M. 2,3-Secoaromadendrane-type sesquiterpenoids (5, 6) from *P. ovalifolia* show anti-tumor activity (2-4 $\mu\text{g/ml}$) against melanoma. Marsupelloic

(7) and acetoxymarsupellone (8) from *Marsupella emarginata* showed antitumor activity (ID₅₀ 1 µg/ml) against P388. Infuscaic acid (10) from *Jungermannia infusca* inhibited the release of superoxide anion radical from guinea pig peritoneal macrophage induced by formyl methionyl leucyl phenylalanine at IC₅₀ 2-7.5 µg/ml. Some liverworts produce sesqui- (9) and diterpene dimers (14) as well as peculiar diterpenoids (11-13).

Bibenzyl derivatives. The thalloid liverwort, *Marchantia polymorpha* can cause allergic contact dermatitis, shows inhibitory activity against Gram-positive bacteria, and has diuretic activity. The methanol extract (100 g) of Japanese *M. polymorpha* was chromatographed on silica gel and Sephadex LH20 to give cyclic bis-bibenzyls, marchantin A(MA) (15, 30g) and its analogues (MB-G). Yield of MA (15) is dependent upon *Marchantia* species. 100 to 120 g of pure MA has been isolated from 2 kg of dried *M. paleacea* var. *diptera*. MA (15) shows various biological activities: cytotoxicity (ED₅₀ 8.29 µg/ml for KB cells), cardiotoxic [increases coronary blood flow (2.5 ml/min. at 0.1 mg)], antimicrobial [against *Acinetobacter calcoaceticus* (MIC 6.25 µg/ml), *Bacillus cereus* (12.5), *B. megaterium* (25), *B. subtilis* (25), *Cryptococcus neoformans* (12.5), and *Staphylococcus aureus* (3.13-25)] and antifungal [against *A. niger* (MIC 25-100 µg/ml), *Piricularia oryzae* (12.5), *Rhizoctonia solani* (50), *Saccharomyces cerevisiae*, and *Trichophyton mentagrophytes* (3.13)], 5-lipoxygenase inhibitory [(89% at 10⁻⁵ mol, 94% at 10⁻⁶ mol, 45% at 10⁻⁷ mol, 16% at 10⁻⁸ mol) against LTB₃ (=5S,12R-dihydroxy-6,8,10,14-eicosatetraenoic acid)], (99% at 10⁻⁵ mol, 97% at 10⁻⁶ mol, 70% at 10⁻⁷ mol, 40% at 10⁻⁸ mol) against 5-HETE (=5-hydroxy-6,8,11,14-eicosatetraenoic acid)] and calmodulin inhibitory activity [ID₅₀ 1.85 µg/ml]. MA (15) and the related cyclic bis-bibenzyls are structurally similar to bis-bibenzylisoquinoline alkaloids such as *d*-tubocurarine (17) which are muscle relaxing active drugs. Surprisingly, MA (15) and its trimethyl ether (16) also had muscle relaxing activity. Nicotine in frog Ringer solution effects maximum contraction of rectus abdominis in frogs (RAF) at a concentration of 10⁻⁶M. After preincubation of 15 or 16 (at a concentration of 2 x 10⁻⁷~2 x 10⁻⁴M) in Ringer solution, nicotine (10⁻⁸~10⁻⁴M) was added. At a concentration of 10⁻⁶ M, the contraction of RAF decreased by about 30%. *d*-Tubocurarine (17) exhibits similar effects as does (15) using acetyl choline and the same results as described above obtained. Although the mechanism of action of 15 and 16 in effecting muscle relaxation is still unknown, it is interesting that



these cyclic bis-bibenzyls from liverworts possessing no nitrogen atoms cause concentration dependent decrease of contraction of RAF. Compounds **15** and **16** also had muscle relaxing activity *in vivo* in mice. MM2 calculations indicate that the conformation of **15** and **16** and the presence of an *ortho* hydroxyl group in **15** and an *ortho* methoxyl group in **16** contribute to the muscle relaxation activity. The thalloid liverwort, *Blasia pusilla* produces cyclic bis-bibenzyl dimers (**18-21**)⁵ which shows plant growth inhibitory activity. *Plagiochila sciophila* elaborates plagiochine A (**22**) which showed neuritic sprouting and enhancement of choline acetyl transferase activity at 10⁻⁶ M. Radulanin K (**23**) from *Radula javanica* inhibited the release of superoxide anion radical from guinea pig macrophage at IC₅₀ 6μg/ml.

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