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TRITERPENOID TAXONOMIC MARKERS FOR *STEMONOPORUS* AND OTHER GENERA OF THE DIPTEROCARPACEAE*

WICKRAMASINGHE M. BANDARANAYAKE†, SUBADRA KARUNANAYAKE†, SUBRAMANIAM SOTHEESWARAN†, M. UVAIS S. SULTANBAWA†‡ and SINNATHAMBY BALASUBRAMANIAM§

†Department of Chemistry, University of Sri Lanka, Peradeniya Campus, Peradeniya, Sri Lanka; §Department of Botany, University of Sri Lanka, Peradeniya Campus, Peradeniya, Sri Lanka

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Abstract—The bark and/or timber extracts of *Stemonoporus affinis, S. cordifolius, S. elegans, S. lancifolius, S. oblongifolius* and *S. petiolaris* have been studied. The following compounds have been isolated: α-amyrin, δ-amyrenone, ursolic acid, sitosterol, sitosteryl-*o*-methoxybenzoate, ursonic acid, acetylursolic acid, 4-hydroxybenzaldehyde, methyl 2,4-dihydroxybenzoate, 2α-hydroxyursolic acid and bergenin. TLC examination of extracts of *S. petiolaris, S. canaliculatus* and *S. reticulatus* is reported. Triterpenoid taxonomic markers are summarized for *Dipterocarpus, Doona, Shorea* and *Stemonoporus*.

INTRODUCTION

In Sri Lanka, 44 of the 45 species in the family Dipterocarpaceae are endemic. These species are distributed among the following seven genera [1]. *Cotylelobium* Pierre, *Dipterocarpus* Gaertn. f., *Hopea* Roxb., *Shorea* Roxb. ex Gaertn. f., *Stemonoporus* Thw., *Vateria* L., *Vatica* L. of which the endemic genus *Stemonoporus* contains 15 species. No chemical investigation has hitherto been done on this latter genus, though Bisset and co-workers have examined the resins of *Doona* Thw. [2], and *Dipterocarpus* [3] from Sri Lanka along with the resins of other species of Dipterocarpaceae from other South East Asian countries. The chemotaxonomic studies on Dipterocarpaceae have been based mostly on the chemical constituents of the resin [2–13] though some studies have also been conducted on the bark and timber of a few species [14–16]; one or more triterpenes belonging to the dammarane series are generally present. In this study the bark and timber of eight species of *Stemonoporus* have been examined.

RESULTS AND DISCUSSION

The details of the species of *Stemonoporus* examined and the compounds isolated are given in Table 1. As in all other members of the Diptrerocarpaceae, most of the compounds isolated from the genus *Stemonoporus* were triterpenes. The most outstanding feature of the chemistry of the genus is the absence of the tetracyclic triterpenes possessing the dammarane skeleton, which are otherwise widespread in the family.

The triterpenes isolated from the *Stemonoporus* species belong only to the ursene or oleanene series. All the species examined had the pentacyclic triterpenes δ-amyrenone, α-amyrin and ursolic acid, sitosterol and sitosteryl-*o*-methoxybenzoate; this is the first report of this ester as a natural product.

β-Amyrin, present in most species of *Doona* and *Shorea* [5, 15, 16], is absent, This fact and the presence of δ-amyrenone in all species of *Stemonoporus* examined could be attributed to the possible presence of an active dehydrogenase enzyme system specific to the oleanane skeleton.

De Silva *et al.* [14] reported the isocoumarin bergenin for the first time from Dipterocarpaceae. Bark extracts of five *Stemonoporus* species growing in the warm lowland forest was shown to have bergenin in large concentrations. By contrast, the bark extractives of the *Stemonoporus* species growing in the cool montane forest lack bergenin. One species, *Stemonoporus elegans* growing in the Adams peak wilderness (montane forest) did not have bergenin in its bark extractives but instead the timber extractives had two other aromatic compounds namely, 4-hydroxybenzaldehyde and methyl 2,4-dihydroxybenzoate.

α-Amyrin and δ-amyrenone have so far not been isolated from any species of Dipterocarpaceae. These two along with ursolic acid, present in all the species of *Stemonoporus* studied, can be considered to be the triterpenoid markers for this endemic genus.

The available data on triterpenoid distributions in the Dipterocarpaceae are collected in Table 2. Each genus has a different set of compounds.

Dipterocarpus, Doona and *Shorea* have a common feature in that at least two of the compounds possess the dammarane skeleton. *Doona*, like *Stemonoporus*, was

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‡ For correspondence.

Table 1. Terpenoids and phenolics of *Stemonoporus* species*

Compounds isolated	*S. affinis* Thw.		*S. cordifolius* (Thw.) Alston		*S. elegans* (Thw.) Alston		*S. lancifolius* (Thw.) Ashton	
	Bark	Timber	Bark	Timber	Bark	Timber	Bark	Timber
Sitosteryl ester	0.003	0.002	0.003	—	0.006	0.002	0.005	0.007
Sitosterol	0.016	0.106	0.025	0.150	0.080	0.015	0.095	0.214
δ-Amyrenone	0.004	0.002	0.005	0.001	0.003	0.003	0.002	0.008
α-Amyrin	0.031	0.062	0.039	0.105	0.040	0.010	0.019	0.029
Ursolic acid	0.040	0.015	0.015	0.009	0.012	0.055	0.027	0.086
Ursonic acid	0.012	0.003	—	—	—	—	0.003	0.010
Acetylursolic acid	—	—	—	—	0.020‡	—	—	—
2α-Hydroxyursolic acid	—	—	0.020	—	—	—	—	—
4-Hydroxybenzaldehyde	—	—	—	—	—	0.005	—	—
Methyl 2,4-dihydroxybenzoate	—	—	—	—	—	0.006	—	—
Bergenin	2.6	—	—	—	—	—	1.8	—

	S. oblongifolius (Thw.)	*S. petiolaris* (Thw.)		*S. canaliculatus* (Thw.)		*S. reticulatus* (Thw.)	
	Bark	Bark	Timber†	Bark†	Timber†	Bark†	Timber†
Sitosteryl ester	0.012	—	+	+	+	+	+
Sitosterol	0.290	0.052	+++	+++	+++	+++	+++
δ-Amyrenone	0.002	0.002	+	+	+	+	+
α-Amyrin	0.204	0.023	+++	+++	+++	+++	+++
Ursolic acid	0.019	0.020	++	++	++	++	++
Ursonic acid	—	0.084	++	—	+	+	+
Acetylursolic acid	0.109	—	++	—	—	—	—
2α-Hydroxyursolic acid	—	—	—	—	—	+	+
4-Hydroxybenzaldehyde	—	—	—	—	—	—	—
Methyl 2,4-dihydroxy benzoate	—	—	—	—	—	—	—
Bergenin	—	3.5	—	+++++	—	+++++	—

* Expressed as a % of the dry weight of the plant part. † (TLC analysis) Relative intensities within a given species are indicated by the + sign. ‡ Mixture of acetylursolic and acetyloleanolic acid.

at one time regarded as a genus endemic to Sri Lanka. Ashton [1] in a recent study of the Dipterocarpaceae has however submerged the genus *Doona* into *Shorea* but the chemotaxonomic data presented in Table 2 suggest that the two genera should be kept distinct [17].

EXPERIMENTAL

Bark and timber of the *Stemonoporus* species were obtained as indicated below: *S. affinis*—Rangala, Sri Lanka (sub-montane); *S. cordifolius*—Kotiyagala, Sri Lanka (montane forest); *S. canaliculatus* and *S. reticulatus* both from Kanneliya (wet lowland forest) in the south of Sri Lanka; *S. elegans*—Adams Peak wilderness; *S. lancifolius*—Kitulgala (wet lowland forest); *S. oblongifolius*—Rajamale, Sri Lanka (montane forest); *S. petiolaris* Gillimale (wet lowland forest). General procedures have been given in earlier parts. The $CHCl_3$ and MeOH extracts were used for TLC examination. Compounds have been shown to be identical with authentic samples by mmp, IR and TLC comparison.

Sitosteryl-o-methoxybenzoate. Petrol or C_6H_6 extracts of all the species, when separated on a column of Si gel, gave the same pure ester on elution with petrol–C_6H_6 (4:1). The ester had mp 78–80°, $[\alpha]_D^{26}$ +41° ($CHCl_3$), R_f 0.36 (C_6H_6–petrol 1:9), IR ν_{max}(nujol) 1745, 1610, 1270, 1250, 1220, 1205, 1180, 1020, 965, 930, 740 and 735 cm^{-1}. MS *m/e* 548(M^+ 100%), 536(2), 534(2), 409(5), 389(17), 381(31), 369(15), 341(15), 308(32), 285(31), 271(16), 267(13), 258(33), 239(15), 229(31), 218(29). NMR τ ($CDCl_3$, 60 MHz) 2.78 (aromatic), 6.0 (OMe), 7.8 and 8.72 (Me and CH_2). Hydrolysis of the ester (0.01 g) gave sitosterol (0.008 g), mp 136–7°, $[\alpha]_D^{26}$ −34.5° ($CHCl_3$) (lit., [18], 137°,

Table 2. Triterpenoid chemotaxonomic markers of the Dipterocarpaceae genera

Genera*	Triterpenoid chemotaxonomic markers†	References
Dipterocarpus (41)	Dammarenediol 20S, Dipterocarpol, Dammaradienone	[3, 16]
Doona (6)	Dammarenediol 20S, Hydroxydammarenone 20S, ψ-Taraxasterol, β-Amyrin	[2, 16]
Shorea (37)	Dammarenediol 20S, Dipterocarpol, Shoreic acid, Dammarenolic acid, Ursolic aldehyde, β-Amyrin	[5]
Stemonoporus (8)	δ-Amyrenone, α-Amyrin, Ursolic acid	This paper

* Numbers in parenthesis refer to the numbers of species for which data are available. † Only compounds present in more than 70% of the species examined of a particular genus are considered as markers. And only those genera where more than 5 species have been studied are included. Most of the work for *Dipterocarpus*, *Doona* and *Shorea* is on the chemical composition of the resins.

$[\alpha]_D$ −36°) and *o*-methoxybenzoic acid (0.002 g), mp 97° (from Me_2CO), (lit. [19] 101°), identical with an authentic sample.

δ-Amyrenone. Continued elution of the column gave δ-amyrenone mp 198° (MeOH–Me_2CO), $[\alpha]_D^{26}$ −20° ($CHCl_3$) (lit., [18] 198–201° $[\alpha]_D$ −12°), R_f 0.44 (C_6H_6). δ-Amyrenone isolated from all the species was identical with an authentic sample.

α-Amyrin. Elution of the column with C_6H_6–petrol (1:3) gave in all the cases examined, α-amyrin as crystals. Mp 182–4° (MeOH), $[\alpha]_D^{26}$ +82° ($CHCl_3$) (lit., [18] 186°, $[\alpha]_D$ +83.5°), R_f 0.50 ($CHCl_3$), identical with an authenetic sample.

Sitosterol. Elution of the column with C_6H_6 gave sitosterol mp 136–7°.

Ursonic acid. The Na_2CO_3 soluble fraction when chromatographed over Si gel and eluted with $CHCl_3$, gave ursonic acid as crystals mp 248° (petrol), $[\alpha]_D^{26}$ +90° ($CHCl_3$) (lit. [20] 270–5°, $[\alpha]_D$ +80°), R_f 0.46 (MeOH–$CHCl_3$, 1:19), identical with an authentic sample. Ursonic acid was found in the bark and timber extracts of *S. affinis, S. lancifolius, S. petiolaris, S. reticulatus* and in the timber extracts of *S. canaliculatus.*

Ursolic acid. The acidic fractions of all the species of *Stemonoporus* on column chromatographic separation over Si gel and elution with $CHCl_3$–MeOH (99:1) gave ursolic acid mp 294° (petrol), $[\alpha]_D^{26}$ +68.3° (lit., [18] 291°, $[\alpha]_D$ +66°), R_f 0.40 (MeOH–$CHCl_3$, 1:9), identical with an authentic sample.

Bergenin. Bark MeOH extracts of *S. affinis, S. lancifolius,* and *S. petiolaris* on cooling deposited crystals of bergenin mp 142–5° (MeOH), $[\alpha]_D^{26}$ −41° (MeOH) (lit., [21] 133° (hydrated) $[\alpha]_D$ −47°), R_f 0.25 (MeOH–$CHCl_3$ 7:93). Bergenin isolated was dried at 150° for 15 min. to yield anhydrous bergenin, mp 232–4° (lit. [21] 234°), identical with an authenetic sample. Bergenin was shown to be present in the bark extractives of *S. canaliculatus* and *S. reticulatus* by TLC.

Acetylursolic acid. The bark extracts of *S. oblongifolius* on elution from a Si gel column with $CHCl_3$–C_6H_6 (4:1) gave pure acetylursolic acid mp 288° (C_6H_6), $[\alpha]_D^{26}$ +60° ($CHCl_3$) (lit., [18] 289–92°, $[\alpha]_D$ +62.6°), R_f 0.25 (chloroform), identical with an authentic sample. From the bark of *S. elegans* acetylursolic acid was isolated as a mixture with acetyloleanolic acid (both compounds have the same R_f on TLC and similar MS fragmentation). TLC analysis of the timber extracts of *S. petiolaris* also showed the presence of acetylursolic acid, acetyloleanolic acid or a mixture of these two as in *S. elegans.*

4-Hydroxybenzaldehyde and Methyl 2,4,-dihydroxy benzoate. Timber extract of *S. elegans* with C_6H_6 was first separated into acidic and neutral fractions. Neutral fraction when chromatographed over Si gel and elution with $CHCl_3$–C_6H_6 (3:1) gave 4-hydroxybenzaldehyde mp 114° (C_6H_6) (lit. [19] 115°), R_f 0.28 ($CHCl_3$), identical with an authentic sample. Further elution with $CHCl_3$ gave methyl 2,4-dihydroxybenzoate mp 110° (lit. [18] 118–119°), R_f 0.30 ($CHCl_3$). It gave an acetate mp 185° (lit. [18] 187°).

2α-Hydroxyursolic acid. The bark extract of *S. cordifolius* after separating the nonacidic fraction and column chromatographic separation (Si gel, $CHCl_3$–MeOH, 19:1) gave 2α-hydroxyursolic acid, as a white solid mp 249° (MeOH), $[\alpha]_D^{26}$ +43° (C_5H_5N) (lit. [22] 243–45°, $[\alpha]_D$ +42.1°), R_f 0.32 (MeOH–$CHCl_3$ 5:95). TLC analyses of the bark and timber extracts of *S. reticulatus* showed the presence of 2α-hydroxyursolic acid.

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REFERENCES

1. Ashton, P. S. (1972) *Blumea* **XX**, 2, 357.
2. Diaz, M. A., Ourisson, G. and Bisset, N. G. (1966) *Phytochemistry* **5**, 855.
3. Bisset, N. G., Diaz, M. A., Ehret, C., Ourisson, G., Palmade, M., Patil, F., Pesnelle, P. and Streith, J. (1966) *Phytochemistry* **5**, 865.
4. Diaz, M. A., Ehret, C., Ourisson, G., Palmade, M., Patil, F., Pesnelle, P. and Streith, J. (1966) *Vietnamica Chim. Acta* 78.
5. Bisset, N. G., Chavanel, V., Lantz, J. and Wolff, R. E. (1971) *Phytochemistry* **10**, 2451.
6. Cheung, H. T. and Tan, T. C. (1972) *Australian J. Chem.* **25**, 2003.
7. Bisset, N. G., Diaz-Parra, M. A., Ehret, C. and Ourisson, G. (1967) *Phytochemistry* **6**, 1395.
8. Hirose, Y., Yanagawa, T., Sayana, T., Igarishi, I. and Nakatsuka, T. (1968) *J. Japan Wood Res. Soc.* **14**, 36.
9. Hirose, Y., Yanagawa, T. and Nakatsuka, T. (1968) *ibid.*, 59.
10. Yanagawa, T., Hirose, Y. and Nakatsuka, T. (1968) *ibid*, 440.
11. Cheung, H. T. and Fang, M. C. (1968) *J. Chem Soc.* (*C*), 1047.
12. Cheung, H. T. (1968) *J. Chem. Soc.* (*C*), 2686.
13. Cheung, H. T. and Wong, C. S. (1972) *Phytochemistry* **11**, 1771.
14. De Silva, L. B., Rodrigo, S., Wijesekera, R. O. B. (1964) *Proc. International Symp. of Medicinal Plants* 133.
15. Gunawardana, Y. A. G. P. and Sultanbawa, M. U. S. (1974) *Proc. Ceylon Assoc. Adv. Sci.* 118; (1975) unpublished results.
16. Bandaranayake, W. M., Gunasekera, S. P., Karunanayake, S., Sotheeswaran, S. and Sultanbawa, M. U. S. (1975) *Phytochemistry* **14**, 2043.
17. Thwaites, G. H. K. (1864) *Enumaratio Plantarum Zeylaniae.* Dulau and Co, London; Thwaites, G. H. K. (1854) *Hookers J. Bot.* **6**, 67.
18. *Dictionary of OrganicCompounds* (1965) **5**, 2902; **1**, 228 and 229; **5**, 3231. Eyre and Spottiswoode Ltd., London.
19. Mann, F G. and Saunders, B. L. *Practical Organic Chemistry* (1960) Longmans & Green, London.
20. Mills, J. S. and Werner, A. E. A. (1955) *J. Chem. Soc.* 3132.
21. Hay, J. E. and Haynes, C. J. (1958) *J. Chem. Soc.* 2231.
22. Glen, A. T., Laurie, W., McLean, J. and Younes, M. El-G. (1967) *J. Chem. Soc.* (*C*), 510.