Phytochemistry, Vol. 24, No. 9, pp. 1991–1993, 1985.
Printed in Great Britain.

0031-9422/85 $3.00 + 0.00

ELLAGIC ACID DERIVATIVES FROM *RHABDODENDRON MACROPHYLLUM**

WILSON WOLTER FILHO, ARNALDO I. DA ROCHA,† MASSAYOSHI YOSHIDA and OTTO R. GOTTLIEB‡

Instituto Nacional de Pesquisas da Amazônia, Conselho Nacional de Desenvolvimento Científico e Tecnológico, 69000 Manaus, AM;
‡Instituto de Química, Universidade de São Paulo, 05508 São Paulo, SP, Brazil

(*Received* 15 *November* 1984)

Key Word Index—*Rhabdodendron macrophyllum*; Rhabdodendraceae; oleanolic acids; 24-methylenecycloartenol; ellagic acids.

Abstract—*Rhabdodendron macrophyllum* contains 24-methylenecycloartenol in the leaves, as well as 3-*O*-acetylaleuritolic acid and glycosides of oleanolic acid, hederagenin and arjunolic acid in the branches. Accompanied by 5′-methoxy-3,4,3′-tri-*O*-methylflavellagic acid, 3,4,3′-tri-*O*-methylflavellagic acid and ellagic acid (possibly in the form of methyl ethers), such saponins also occur in the roots. Chemically the Rhabdodendraceae would fit into the Rosiflorae or even better into the Myrtiflorae.

INTRODUCTION

A critical analysis of the proposed morphological alignments led Cronquist [1] to conclude that the Rhabdodendraceae, with the sole genus *Rhabdodendron*, should be regarded as a distinct family [2] in the order Rosales *s. lat.* This point of view is at variance with previous authors who had placed the Rhabdodendraceae in other orders.

The genus *Rhabdodendron* consists of two established species native to tropical South America, *R. macrophyllum* (Spr. ex Benth.) Huber and *R. amazonicum* (Spr. ex Benth.) Huber. A third species *R. gardneranum* (Benth.) Sandw. is only known from the type collection [3]. The present paper describes a phytochemical analysis of *R. macrophyllum*. A report on *R. amazonicum* will follow shortly. It is hoped that the chemical data will illuminate the problem of accommodation of the family into an appropriate order.

RESULTS

Leaves of *R. macrophyllum* were found to contain 24-methylenecycloartenol (**1**) and a mixture of glycosides which yielded oleanolic acid (**2**) upon hydrolysis. Branches contain 3-*O*-acetylaleuritolic acid (**3**) and a mixture of glycosides which gave **2**, hederagenin (**4**) and arjunolic acid (**5**) upon hydrolysis. Roots contain the triterpenes **2** (obtained only after hydrolysis), **3** and **4**, besides several derivatives of ellagic acid. While 3,4,3′-tri-*O*-methylflavellagic acid (**6a**) and 5′-methoxy-3,4,3′-tri-*O*-methylflavellagic acid (**6b**) were actually isolated from the extract, tetra-*O*-methylellagic acid (**7**) was obtained only after methylation of a mixture of partially *O*-methylated ellagic acids. These ethers and perhaps even free ellagic acid may thus be the true constituent(s) of the plant.

Physical constants and spectra of the triterpenes **1** [4, 5], **2** [4, 6, 7], **4** [4, 6, 8] and **5** [4, 6, 8] and of their acetates were fully consistent with reported data. Precisely the same method was used for the identification of **6a** [9, 10] and, with exception of the derivatization into acetate, for **3** [11] and **7** [9, 12].

Although **6b** is a new compound, it was immediately recognized as a methoxylated derivative of **6a** by mass ($[M]^{+\cdot}$ **6b** 390, **6a** 360) and NMR spectrometry. The sole difference in the ^{1}H NMR spectra refers to the replacement of the one-proton singlet in the aromatic region of **6a**-diacetate (δ7.93 in $CDCl_3$) by an additional (fourth) three-proton singlet in the methoxyl region of **6b**-diacetate. Significantly also the UV spectra of **6b** and **6a** are extremely similar (λ_{max} nm 247 ± 2, 361 ± 1^{inf}; 378.5 ± 0.5) even after addition of aluminium chloride (λ_{max} nm 247 ± 2, 385 ± 4^{inf}, 412 ± 2) and sodium acetate (λ_{max} nm 247 ± 2, 270^{inf}, $411 + 1$). This means that not only in **6a**, but also in **6b** one of the hydroxyls must be vicinal to a carbonyl (to allow formation of an aluminium chelate) and the other conjugated with a carbonyl (to allow transitions resulting in a symmetrical extended quinone-methine chromophore [13]). Since the vicinal position of these hydroxyls can be excluded (no $H_3BO_3 + NaOAc$ shifts), only **6b** can represent the compound correctly.

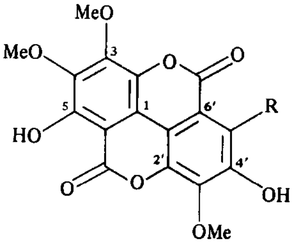
DISCUSSION

Ellagic acid is an excellent systematic marker [14] of the more primitive superorders of the rosiflorean block [15]. *O*-Alkylated derivatives are of a much more restricted occurrence, having been observed chiefly in taxa in which ellagic acid is widespread and abundant [16]. While they have been found sporadically in some scattered rosiflorean families, such as Euphorbiaceae, Lecithydaceae, Tamaricaceae [16] and Vochysiaceae [12]; it is only in the major families of the Myrtiflorae (Combretaceae, Melastomataceae, Myrtaceae, Punicaceae, Rhizophoraceae and Sonneratiaceae) that *O*-methylellagic acids

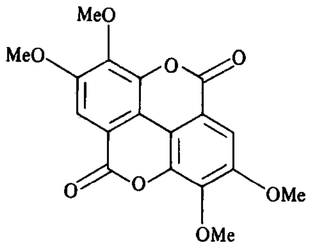
---

*Part 1 in the proposed series "The Chemistry of Brazilian Rhabdodendraceae". Taken from part of the Doctorate Thesis presented by W.W.F. to Universidade de São Paulo (1985).

†Permanent address: Universidade do Amazonas, 69000 Manaus, AM, Brazil.



6a R = H
6b R = OMe



7

appear to constitute a definite systematic character [16].

In view of its chemical profile alone, *Rhabdodendron* can be separated from two of the families with which it has been associated in the past, the Phytolaccaceae [2] and the Rutaceae [17–19]. The former, as their order, the Caryophyllales, belong to the magnolialean block [15] and lack ellagic acids; the latter, as their order, the Rutales, contain anthranilic acid-derived alkaloids [20] and limonoids [21], compound types which are absent from Rhabdodendraceae. The alignment of this family with the Rosales *s. lat.* [1] is more consistent with the data. Indeed authors of the past century had already referred the then known species of the group to the Chrysobalanaceae [1, but see 2] and di-*O*-methylellagic acid occurs in the Fagaceae [16], two families belonging to the Rosiflorae. Dahlgren considers the strong affinity of this superorder with the Myrtiflorae [22], where it would be appropriate to include the Rhabdodendraceae on the basis of the present chemical data.

EXPERIMENTAL

Isolation of the constituents. Plant material was collected near Igarapé do Tarumã, Manaus, AM, and identified by Dr. William A. Rodrigues (Voucher: INPA Herbarium no. 50.379). *Leaves*: dry powder (3.6 kg) was extracted with EtOH (Soxhlet). The extract (13 g) was filtered though silica gel (60 g), washed successively with C_6H_{14}, Et_2O, EtOAc and MeOH, giving respectively fractions of 30, 2290, 707 and 4200 mg. The Et_2O-fraction was submitted to column chromatography (silica gel, 70 g). Elution was performed with C_6H_{14}–C_6H_6 8:2 (16 × 75 ml), C_6H_6 (25 × 75 ml), $CHCl_3$ (5 × 75 ml). Evaporation of C_6H_6 gave **1** (874 mg). The MeOH-fraction was hydrolysed in acid medium [23]. The aglucon (1.4 g) was crystallized from MeOH to **2** (1 g). *Branches*: dry powder (0.5 kg) was extracted with EtOH (Soxhlet). The extract (26 g) was partially soluble in C_6H_{14} (1.2 g) and Et_2O (4.4 g). The latter fraction was submitted to column chromatography (silica gel, 60 g). Elution was performed with C_6H_{14} (11 × 50 ml), C_6H_6 (23 × 50 ml), C_6H_6–Me_2CO (9:1, 8 × 50 ml). Evaporation of the two latter eluates gave **3** (1.9 g). The insol. portion of the extract (20 g) was hydrolysed as described above. The aglucon (6.3 g) was submitted to column chromatography (silica gel 190 g). Elution was performed with C_6H_6 (18 × 100 ml), $CHCl_3$ (16 × 100 ml), EtOAc (15 × 100 ml), Me_2CO (8 × 100 ml). The latter three eluates gave in order **2** (3.9 g), **4** (80 mg) and **5** (380 mg). *Roots*: dry powder (1.4 kg) was extracted with EtOH (Soxhlet). The extract (76 g) was washed successively with C_6H_{14}, Et_2O, EtOAc and Me_2CO. The C_6H_{14}-soluble portion (4 g) was crystallized repeatedly from MeOH to **3**. The Et_2O-soluble portion (3.6 g) also gave **3** (3 g). The EtOAc-soluble portion (1.6 g) was submitted to column chromatography (silica gel 45 g). Elution was performed with EtOAc (50 × 30 ml frs.) Frs. 9–19 gave **4** (76 mg) and frs. 41–48 gave **6** (157 mg). Upon concentration of the Me_2CO-washings a precipitate (7.4 g) appeared. This was filtered and extracted with hot dioxane. The filtered soln. gave crystals of **7** (932 mg). The insoluble portion (49 g) of the original EtOH-extract was hydrolysed as described above. The aglucone (29.3 g) was washed successively with Et_2O and EtOAc. The Et_2O-soluble portion (4.5 g) gave **2** (3 g). The insoluble portion (23.5 g) was, in part, methylated with CH_2N_2. The crude product was crystallized from C_6H_6 to **8**.

5'-Methoxy-3,4,3'-tri-O-methylflavellagic acid (**6b**). Mp > 300° (dioxane). IR ν_{max}^{KBr} cm^{-1}: 3310, 3200, 1740, 1670, 1635, 1590, 1518, 1475, 1446, 1410, 1350, 1290, 1195, 1150, 1090, 1065, 1000, 957, 932, 860. λ_{max}^{EtOH} nm (ε): 249 (27880), 362 (6100), 379 (3600); $\lambda_{max}^{EtOH+AlCl_3}$ nm: 249, 389, 409; $\lambda_{max}^{EtOH+NaOH}$ nm: 245, 270, 412; no H_3BO_3 + NaOAc shift. ^1H NMR (60 MHz, $CDCl_3$): δ10.5 (*br*, 2OH), 4.25 (*s*, ArOMe), 4.10 (*s*, ArOMe), 4.03 (*s*, 2ArOMe). MS *m/z* (rel. int.): 390 $[M]^{+\cdot}$ (100), 375 (84), 362 (5), 360 (24), 347 (16), 346 (10), 345 (20), 332 (28), 317 (20), 289 (25), 233 (72). *Diacetate*, mp 153–154° (MeOH). IR ν_{max}^{KBr} cm^{-1}: 1785, 1750, 1610, 1590, 1565, 1470, 1360, 1300, 1280, 1180, 1140, 1060, 1040, 960, 930, 875, 845. ^1H NMR (60 MHz, $CDCl_3$): δ4.20 (*s*, ArOMe), 3.98 (*s*, 2ArOMe), 3.91 (*s*, ArOMe), 2.46 (*s*, 2ArOAc). ^1H NMR (60 MHz, C_6D_6): δ3.81, 3.76, 3.71, 3.63, (4*s*, 4ArOMe), 2.18, 1.88 (2*s*, 2ArOAc). MS *m/z* (rel. int.): 474 $[M]^{+\cdot}$ (3), 432 (22), 418 (5), 402 (3), 390 (100), 375 (60), 360 (25), 345 (14), 317 (9), 233 (29). *Monomethyl ether*, mp > 300° (MeOH). ^1H NMR (60 MHz, $CDCl_3$): δ10.53 (*s*, ArOH), 4.30 (*s*, 2ArOMe), 4.06 (*s*, 3ArOMe).

Acknowledgements—O.R.G. is grateful to Prof. Dr. Fritz Ehrendorfer, Botanisches Institut, Universität, Wien, for calling his attention to the problem. Financial aid by FAPESP and FIPEC/BB is acknowledged.

REFERENCES

1. Cronquist, A. (1983) *Nord. J. Bot.* **3**, 75.
2. Prance, G. T. (1968) *Bull. Jard. Bot. Natl. Belg.* **38**, 127.
3. Nelson, B. W. and Prance, C. T. (1984) *Acta Amazon.* **14** (in press).
4. Wehrli, F. W. and Nishida, T. (1976) *Fortschr. Chem. Org. Naturst.* **36**, 88.
5. Shimizu, M. and Ohta, G. (1960) *Chem. Pharm. Bull.* **8**, 108.
6. Halsall, T. G. and Aplin, R. T. (1964) *Fortschr. Chem. Org. Naturst.* **22**, 153.
7. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
8. Cheung, H.-T. and Feng, M.-C. (1968) *J. Chem. Soc.* 1048.
9. Row, L. R. and Raju, R. R. (1967) *Tetrahedron* **23**, 879.

10. Reddy, K. R. S., Srimannarayana, G. and Rao, N. V. S. (1974) *Curr. Sci.* **43**, 544.
11. Carpenter, R. C., Sotheeswaran, S. and Sultanbawa, M. U. S. (1980) *Org. Magn. Reson.* **14**, 462.
12. Corrêa, D. B., Birchal, E., Aguillar, J. E. V. and Gottlieb, O. R. (1975) *Phytochemistry* **14**, 1138.
13. Scott, A. I. (1964) *Interpretation of the Ultraviolet Spectra of Natural Products*, p. 145. Pergamon Press, Oxford.
14. Bate-Smith, E. C. (1984) *Phytochemistry* **23**, 945.
15. Kubitzki, K. and Gottlieb, O. R. (1984) *Taxon* **33**, 375.
16. Lowry, J. B. (1968) *Phytochemistry* **7**, 1803.
17. Huber, J. (1909) *Bol. Mus. Goeldi* **5**, 424.
18. Puff, C. and Weber, A. (1976) *Plant Syst. Evol.* **125**, 195.
19. Takhtajan, A. (1980) *Bot. Rev.* **46**, 225.
20. Gottlieb, O. R. (1982) *Micromolecular Evolution, Systematics and Ecology*, p. 78. Springer, Berlin.
21. Silva, M. das G. F. da, Gottlieb, O. R. and Dreyer, D. L. (1984) *Biochem. Syst. Ecol.* **12**, 299.
22. Dahlgren, R. (1980) *Bot. J. Linn. Soc.* (*London*) **80**, 91.
23. Yadav, N., Misra, G. and Nigam, S. K. (1976) *Planta Med.* **29**, 177.