

CONSTITUENTS OF *HORTIA REGIA*: 6,7-DIMETHOXYCOUMARIN, RUTAECARPINE, SKIMMIANINE, AND (+)-METHYL (*E,E*)-10,11-DIHYDROXY-3,7,11-TRIMETHYL-2,6-DODECADIEENOATE

HELEN JACOBS, FRANK RAMADAYAL,

Centre for National Products Chemistry, University of Guyana, Georgetown, Guyana

STEWART MCLEAN,* MARION PERPICK-DUMONT, FRANK PUZZUOLI, and WILLIAM F. REYNOLDS

Department of Chemistry, University of Toronto, Toronto, Canada M5S 1A1

The genus *Hortia* Vand. (Rutaceae) comprises about 12 species and is confined to South America. *H. arborea* (1), *H. badinii* (2), *H. longifolia* (3), and *H. Braziliiana* (4) have received previous phytochemical attention. *Hortia regia* Sandwith., a canopy tree (30 m tall), is a species endemic to Guyana where it is a food plant ("wild orange") (5).

We have reported previously on the isolation from *H. regia* of the chromene methyl 5-methoxy-2,2-dimethyl-1-2*H*-benzopyran-6-propanoate (6) and the novel tetranortriterpenoid guyanin (7). Continuing our investigation of the roots of the plant, we have also isolated 6,7-dimethoxycoumarin, rutaecarpine, skimmianine, and (+)-methyl (*E,E*)-10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienoate (**1**). The characterization of **1** illustrates the application of 2D-nmr spectroscopy to structural studies.

Compound **1** was isolated as an oily substance, C₁₆H₂₈O₄, [α]_D+17.4°; from a routine spectroscopic examination of the material we concluded that it was a dihydroxylated methyl farnesate closely related to the insect juvenile hormones. In order to establish the structure unequivocally, we employed 2D-nmr techniques that we have developed recently. First, all direct C-H connectivities were identified by a standard ¹H¹³C shift-correlated experiment (8).

A modified version (9) of our XCORFE pulse sequence (10) was then used to identify indirect connectivities (i.e., C-C-H and C-C-C-H connectivities were identified). Modifications, designed to minimize artifacts, included replacement of the initial ¹H 90° pulse by a TANGO pulse sandwich selective for hydrogens not directly bonded to ¹³C (11) and replacement of ¹³C 180° pulses by composite pulses (12). Two experiments were performed with delay times optimized for ⁿJ_{CH} values of 4 and 8 Hz.* Observed connectivities, summarized in Table 1, are sufficient to establish unequivocally the structure of **1**. Three features deserve comment because they are of significance to the general application of this technique. Firstly, while most ²J_{CH} and ³J_{CH} values fall within fairly well defined limits (13), they do vary with structural features in the molecule (in the case of **1**, connectivities through sp³ or sp² carbons are the significant factors). In many cases, it is possible to choose delay times that are appropriate for a wide range of structural types, but it may be desirable to carry out further experiments with delay times optimized for specific expected ⁿJ_{CH} values. The second feature, illustrating one important advantage of our XCORFE pulse sequence, is that two-bond and three-bond connectivities, ¹H-C-¹³C(H) and ¹H-C-C-¹³C(H), may be distinguished,

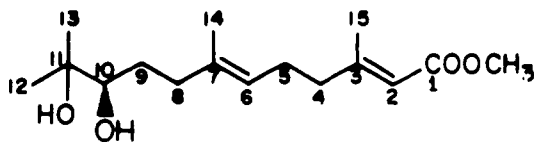


TABLE 1. ^1H - and ^{13}C - Spectral Data for **1** in CDCl_3^a

Carbon	δC^b	δH^b	^1H , ^{13}C Connectivities ^c
1	167.30	—	2, OCH ₃
2	115.40	5.67	4, 15
3	160.03	—	4, 15
4	40.78	2.19	2, 5(s)
5	25.78	2.21	4(s), 6
6	123.64	5.16	5, 8a, 14
7	135.91	—	5, 8a, 14
8	36.64	2.09, 2.25	6, 9b(s), 10, 14
9	29.61	1.40, 1.57	8a(s), 8b(s), 10(s)
10	77.96	3.32	8b, 9a(s), 12, 13
11	73.15	—	10, 12, 13
12	23.01 ^d	1.15	10, 13
13	26.41 ^d	1.19	10, 12
14	15.91	1.62	6, 8a
15	18.79	2.17	2, 4
OCH ₃	50.90	3.68	—

^aObtained on a Varian XL-400 spectrometer using 50 mg of **1** in 0.5 ml of CDCl_3

^b ^{13}C - and ^1H - chemical shifts in ppm from TMS. Direct (1-bond) connectivities.

^cProtons giving peaks at each ^{13}C -frequency in XCORFE experiment. Indirect (2- or 3-bond) connectivities. Label a refers to the high field signal for a non-equivalent CH_2 while (s) indicates that the signal is split by H-H coupling showing ^1H - ^{13}C (H) connectivity.

^dAssignments of C-12 and C-13 could be interchanged.

provided that the ^{13}C is directly bonded to at least one H. In the former, the vicinal ^1H - ^1H coupling (between the protons shown explicitly) is observed on the ^1H frequency axis (see Table 1), but in the latter, the protons are fully decoupled (10). The third feature, often of considerable value in structural studies, is that connectivities through heteroatoms can be observed (e.g., C-O-CH₃).

Although the structure of **1** is not new, we may note that it was established rapidly, unequivocally, and non-destructively by our nmr techniques and without phytochemical clues to its identity. Indeed, it belongs to a class of sesquiterpenoids rarely found in plants and more commonly associated with the insect juvenile hormones. The importance of the juvenile hormones has led to the preparation of their related diols by synthetic and biosynthetic procedures; (\pm)-**1** has been synthesized and studied spectroscopically (14), and both enantiomers

have been identified as products of fungal metabolism (15, 16). The first isolation of ($-$)-**1** from a higher plant, *Cleistopholis patens* (Annonaceae), was reported very recently (17), and this appears to be the only other report of the occurrence of a plant metabolite as closely related to the insect juvenile hormones. From earlier configurational assignments (15-17), it follows that our material, ($+$)-**1**, has the 10R configuration common to the insect juvenile hormones. The phytochemical role of these diols, isolated from two unrelated plant taxa and in both enantiomeric forms, remains a matter for conjecture.

EXPERIMENTAL

Plant material was collected in the forest west of the Mahica river, Demerara, Guyana, and identified as *H. regia* by Mr. C.A. Persaud; voucher specimens are lodged in the University of Guyana Herbarium and in the Institute of Systematic Botany, Utrecht.

Melting points were determined on a Thomas-

Kofler micro hot stage. Optical rotations were measured on a Perkin-Elmer 243B polarimeter. Exact masses were measured on an AEI MS30 high-resolution mass spectrometer. The 2D-nmr spectra were obtained on a Varian XL-400 spectrometer; experimental details are provided elsewhere (9, 10).

EXTRACTION, ISOLATION, AND IDENTIFICATION.—Dried roots (770 g) were ground and extracted by percolation with cold MeOH. Evaporation provided oily material (117 g) that was treated with MeOH-H₂O (9:1) and filtered. The filtrate was exhaustively extracted with hexane, concentrated, and then extracted with CHCl₃. The material (30 g) that was extracted by CHCl₃ was chromatographed (Al₂O₃, activity III, gradient elution with EtOAc/hexane). Material from 20% EtOAc fractions was rechromatographed (SiO₂, gradient elution with EtOAc/hexane). This provided, in order, the chromene (6), 6,7-dimethoxycoumarin, rutaecarpine, and skimmianine. Material from 40% EtOAc fractions was flash chromatographed (SiO₂, Me₂CO-hexane, 1:3) and provided, in order, compound 1 and guyanin. Each constituent was purified further for characterization and identification. (Details are available on request.) 6,7-Dimethoxycoumarin recrystallized (MeOH), mp 143-144°; C₁₁H₁₀O₄ (exact mass 206.0575), ir, uv, ¹H and ¹³C nmr, ms [lit. (18-21)]. Rutaecarpine recrystallized (Me₂CO), mp 258-259°, C₁₈H₁₃N₃O (exact mass 287.1063), ir, uv, ¹H nmr, ms [lit. (22)]. Skimmianine recrystallized (MeOH), mp 175-177°, C₁₄H₁₃NO₄ (exact mass 259.0845), ir, uv, ¹H and ¹³C nmr, ms [lit. (24-27)]. Compound 1 chromatographed (SiO₂ plate, EtOAc-hexane, 1:1), colorless oil, [α]_D+17.4° (c 2.6, CHCl₃), C₁₆H₂₈O₄ (M⁺ v. weak; (M-H₂O)⁺ exact mass 266.1881), ir, uv, ¹H and ¹³C nmr, ms [lit. (14-17)]. Detailed characterization of the chromene (6), colorless oil, and guyanin (7), mp 261-262°, are provided elsewhere.

ACKNOWLEDGMENTS

The Centre at the University of Guyana is grateful for generous support from the Canadian International Development Agency. The research in the Toronto laboratory is supported by grants from the Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

- I.J. Pachter, R.F. Raffauf, G.E. Ulyyot, and O. Ribeiro, *J. Am. Chem. Soc.*, **82**, 5187 (1960).
- D. de B. Corrêa, O.R. Gottlieb, and A.P. de Padua, *Phytochemistry*, **18**, 351 (1979).
- D. de B. Corrêa, O.R. Gottlieb, A.P. de Padua, and A.I. Rocha, *Rev. Latinoam. Quim.*, **7**, 43 (1976).
- I.J. Pachter, R.J. Mohrbacher, and D.E. Zacharias, *J. Am. Chem. Soc.*, **83**, 635 (1961).
- D.B. Fanshawe, "Forest Products of British Guiana," Forestry Bulletin No. 1 3rd edition, Forest Department, British Guiana, 1961, p. 82.
- H. Jacobs, F. Ramadaya, W.F. Reynolds, J. Poplawski, and S. McLean, *Can. J. Chem.*, **64**, 580 (1986).
- H. Jacobs, F. Ramadaya, W.F. Reynolds, and S. McLean, *Tetrahedron Lett.*, **27**, 1453 (1986).
- A. Bax and G.A. Morris, *J. Magn. Reson.*, **42**, 501 (1981).
- M. Perpick-Dumont, M.Sc. Thesis, University of Toronto, 1986.
- W.F. Reynolds, D.W. Hughes, M. Perpick-Dumont, and R.G. Enriquez, *J. Magn. Reson.*, **63**, 415 (1985).
- C. Bauer, R. Freeman, and S. Wimperis, *J. Magn. Reson.*, **58**, 526 (1984).
- M.H. Levitt and R. Freeman, *J. Magn. Reson.*, **33**, 473 (1979).
- P. Hansen, *Prog. Nucl. Magn. Reson. Spectrosc.*, **14**, 175 (1981).
- W. Kuhn and H. Rembold, *Org. Magn. Reson.*, **16**, 138 (1981).
- Y. Suzuki and S. Marumo, *Tetrahedron Lett.*, 1887 (1972).
- K. Imai and S. Marumo, *Tetrahedron Lett.*, 1211 (1976).
- P.G. Waterman and I. Muhammad, *Phytochemistry*, **24**, 523 (1985).
- H. Gunther, J. Prestein, and P. Joseph-Nathan, *Org. Magn. Reson.*, **7**, 339 (1975).
- C.J. Pouchert, "Aldrich Library of FT-IR Spectra," Vol. 2, Aldrich Chemical Co., Milwaukee, 1985, p. 324.
- P. Joseph-Nathan, M. Dominquez, and D.A. Ortega, *J. Heterocyclic Chem.*, **21**, 1141 (1984).
- C.S. Barnes and J.L. Ocolowicz, *Aust. J. Chem.*, **17**, 975 (1964).
- T. Kametani, T. Higa, C.V. Loc, M. Ihara, M. Koizumi, and K. Fukumoto, *J. Am. Chem. Soc.*, **98**, 6168 (1976).
- V. Deulofeu, R. Labriola, and J. De Langhe, *J. Am. Chem. Soc.*, **64**, 2326 (1942).
- J. Holubek and O. Strouf, "Spectral Data and Physical Constants of Alkaloids," Heyden and Son, London, 1965, Card No. 252.
- A.V. Robertson, *Aust. J. Chem.*, **16**, 451 (1963).
- N.M.D. Brown, M.F. Grundon, D.M. Harrison, and S.A. Surgenor, *Tetrahedron*, **36**, 3579 (1980).
- D.M. Clugston and D.B. MacLean, *Can. J. Chem.*, **43**, 2516 (1965).

Received 3 December 1986