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GUYANIN, A NOVEL TETRANORTRITERPENOID. STRUCTURE ELUCIDATION BY 2-D N.M.R. SPECTROSCOPY

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Abstract. The structure of guyanin, a new type of tetranortriterpenoid isolated from *Hortia regia*, has been determined by nmr spectroscopy. Indirect shift correlation experiments were crucial for this determination.

Hortia regia Vand. (Rutaceae), collected in Guyana, has afforded a new substance, guyanin, mp 261 - 2°C, $C_{28}H_{34}O_8$. After a preliminary examination of its nmr spectra indicated that guyanin was the dimethyl ester of a tetranortriterpenoid of an unprecedented structural type, its structure was elucidated entirely by two-dimensional nmr spectroscopy. Structural information from ^1H-^1H couplings is severely limited because protonated carbons are confined in several small isolated units.

A heteronuclear chemical-shift correlation experiment optimized for one-bond ^{13}C-^1H couplings[1] identified all C-H direct connectivities. A similar experiment, but with delay times approximately optimized for geminal ($^{13}\underline{C}$-C-$^1\underline{H}$) and vicinal ($^{13}\underline{C}$-C-C-$^1\underline{H}$) couplings, was used to determine these two- or three-bond connectivities[2]. Cross peaks involving methine or methyl protons were usually clearly observed, but those involving methylene protons were generally weak for two reasons: their signals were complex multiplets and their T_2 relaxation times were short. Consequently, this experiment was repeated with the pulse sequence XCORFE[3] that we recently designed specifically to obtain improved indirect shift-correlated spectra. This sequence maximizes sensitivity by: (i) folding the variable evolution time, t_1, inside the fixed delay, T, used to set up ^1H to ^{13}C polarization transfer, thus minimizing signal loss due to ^1H T_2 relaxation; (ii) providing partial or full decoupling of ^1H multiplets. Spurious peaks associated with direct ^{13}C-^1H connectivities are also eliminated. The XCORFE experiment with guyanin provided more definitive connectivities for methylene protons.

Table: Assigned ^{13}C and ^{1}H Chemical Shifts for Guyanin plus observed hydrogen-carbon connectivities used to establish the structure of Guyanin[a]

Carbon	δ_C[b]	δ_H(m,J)[c]	Hydrogen connectivities[d]
1	176.95	-	3, 25, 26, 27
2	46.30	-	3, 4a,b, 25, 26
3	40.91	3.26(d,10)	4a,b, 24, 25, 26
4	34.33	2.18(d,17), 2.55(dd,10,17)	3
5	173.67	-	3, 4a,b, 28
6	61.95	-	3, 8, 11a, 24
7	208.41	-	(8), 24
8	128.89	5.87(q,1.3)	23
9	175.28	-	(8), 23
10	68.95	-	(3), 8, 12a, 23, 24
11	28.31	2.16(dd,15,6), 2.29(m,15,13,6)	12a,b
12	34.39	1.55(dd,13,6), 1.71(td,13,6)	11b, 22
13	46.57	-	(11a,b), (12a,b), 15, 17, 22
14	173.24	-	11a, 12a, 17, 22
15	117.45	5.90(s)	-
16	164.25	-	15
17	79.95	5.94(d,0.8)	(12b), 22
18	120.85	-	17, 19, 20, 21
19	108.51	6.32(dd,1.7,0.8)	17, 20, 21
20	142.86	7.44(t,1.7)	19, 21
21	140.25	7.56(dt,1.7,0.8)	17, 19, 20
22	18.99	1.16(s)	(12a,b), 17
23	16.37	2.04(d,1.3)	8
24	23.65	1.27(s)	3
25	27.80	0.95(s)	26
26	20.67	1.43(s)	(3), 25
27	51.25	3.52(s)	
28	51.98	3.60(s)	

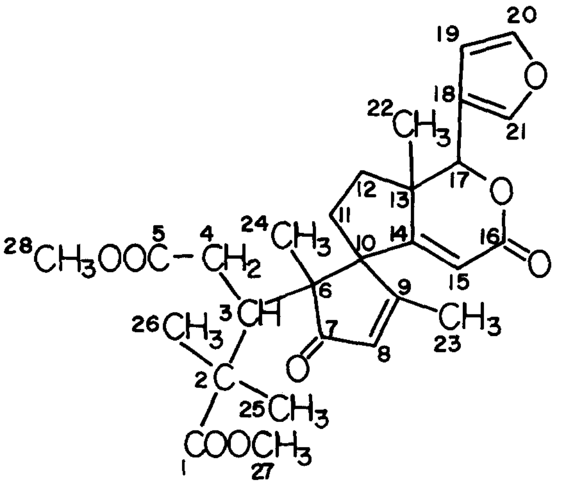
Footnotes to Table

[a] All spectral data were obtained on a Varian XL-400 spectrometer equippted with a 5 mm multinuclear probe. Spectra were obtained for 90 mg of guyanin dissolved in ca 0.5 ml of $CDCl_3$.

[b] ^{13}C chemical shifts in p.p.m. from $(CH_3)_4Si$.

[c] ^{1}H chemical shifts in p.p.m. from $(CH_3)_4Si$ and (in brackets) multiplet pattern and observed values of J_{HH} in Hz.

[d] Hydrogens showing cross-peaks with ^{13}C signals in indirect shift-correlated 2D spectra. In the case of methylene protons, ^{a} is the high field proton. A number in brackets indicates that the observed cross-peak was of low intensity.



Guyanin

[The arbitrary numbering system is used only to identify carbon atoms, and is without any biogenetic implications.]

The results summarized in the Table led us ineluctably to the structure assigned to guyanin. Every possible ^1H to ^{13}C two- or three-bond connectivity (including those of MeO protons) in the side chain gives an observed cross peak, defining this part-structure unambiguously. Not all possible cross peaks are observed for other parts of the molecule (especially where ^{13}C to ^1H couplings are expected to be small), but the data are more than adequate to establish the structure unequivocally. Examination of the Table will show that there is a considerable amount of redundancy in the data. All data are self-consistent and are uniquely in accord with the assigned structure. (Stereochemical assignments can not be made from the present data.)

The indirect shift-correlation technique has been used previously to assign spectra of complex organic molecules[4], but the elucidation of the structure of guyanin, a complex molecule of a new structural type, entirely on the basis of this technique, provides the most telling evidence for its power. Its strength lies in its sensitivity, the extensive information content of the data produced, and its ability to detect connectivities through heteroatoms. This provides distinct advantages over other techniques, such as ^{13}C-^{13}C direct-connectivity experiments[5].

The biogenesis of guyanin is of obvious interest since it requires the oxidative modification of all of the carbocyclic rings of the terpenoid precursor.

The assigned structure has now been confirmed by X-ray crystallography[6].

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