Tetrahedron Letters,Vol.25,No.3,pp 299-302,1984
Printed in Great Britain

0040-4039/84 $3.00 + .00

NEW TOXIC QUASSINOID GLUCOSIDES FROM SIMAROUBA GLAUCA (X-Ray analysis)[1]

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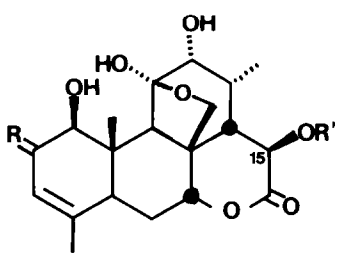
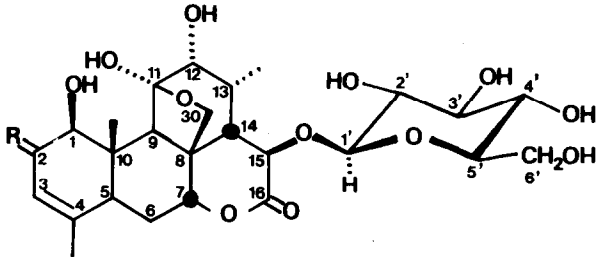
Summary : Two new toxic quassinoid glucosides, 15-O-β-D-glucopyranosyl glaucarubolone 3 and 15-O-β-D-glucopyranosyl glaucarubol 4, have been isolated from Simarouba glauca seeds. Their structures were deduced from spectral data and that of 3 established by single-crystal X-ray analysis.

Recently, we initiated a programme aiming to transform biologically inactive quassinoids into a series of biologically active compounds[2]. For this investigation we required a supply of the inactive glaucarubine 1[3-5]. This quassinoid is known to co-occur with glaucarubinone 2[6] in the Simaroubaceous plant, Simarouba glauca[7,8]. During the isolation procedure of 1 and 2 from the defatted seeds of S. glauca, two new quassinoids were obtained. We now report on the structural elucidation of these compounds which proved to be 15-O-β-D-glucopyranosyl glaucarubolone 3 and 15-O-β-D-glucopyranosyl glaucarubol 4.





1 R = α-OH, β-H ; R' = C(=O)-C(OH)(CH$_3$)-C$_2$H$_5$

2 R = O ; R' = C(=O)-C(OH)(CH$_3$)-C$_2$H$_5$

5 R = O ; R' = H

6 R = α-OH, β-H ; R' = H

3 R = O

4 R = α-OH, β-H

Glaucarubine 1 and glaucarubinone 2 were isolated as previously reported[3,6]. The remaining aqueous solution was treated with an excess of methanol and the voluminous precipitate formed was removed by centrifugation. The supernatant liquid, which showed on TLC the presence of consti-

tuents much more polar than 1 and 2, was concentrated under reduced pressure and extracted several times with n-butanol. Chromatography of the combined n-butanol extracts on Kieselgel 60 (Merck) using the lower phase of a mixture $CHCl_3/MeOH/H_2O$, 65:35:10 as an eluent afforded the glucosides 3 and 4.

Quassinoid 3 crystallized from methanol, m.p. 252-254°C, $[\alpha]_D^{22}$-25.7° (c=1.01 ; pyridine). The molecular formula ($C_{26}H_{36}O_{13}$) was supported by fast atom bombardment (FAB) mass spectrometry which showed the MH^+ peak at m/z 557. A positive anthrone test as well as the presence of abundant fragmentation ions at m/z 394 ($M^{+\bullet}$-162) in the EI and at m/z 395 (MH^+-162) in the FAB mass spectra indicate the presence of a hexose moiety. The i.r. spectrum (nujol) showed hydroxyl absorption at 3325 cm^{-1} and carbonyl bands at 1720 and 1660 cm^{-1} and, in agreement with the formulation of ring A as in 3, the u.v. spectrum showed a maximum at 240 nm (ε 11,185) and the EI mass spectrum the characteristic ions at m/z 151 and 135[7]. The 400 MHz ^1H n.m.r. spectrum (Table) showed the presence of one tertiary, one secondary and one vinyl methyl group. Extensive double resonance experiments allowed the unambiguous assignments of all other protons with the exception of four attributed to the hexose unit (H-2', H-3', H-4' and H-6' B) which give rise to a complex pattern of overlapped signals. Interpretation of these data suggested the aglycone to be glaucarubolone 5. The ^{13}C n.m.r. spectra of quassinoids 1 and 2 have been reported[9] and referring to these results the carbon resonances of the quassinoid 3 could be assigned (Fig. 1) ; the ^{13}C multiplicities were determined by off-resonance decoupling and also by the J-modulated spin-echo technique[10]. The ^{13}C spectral data fully support the glaucarubolone structure of the aglycone.

Unequivocal proof for the structure and the stereochemistry of the quassinoid glucoside 3 was provided by single-crystal X-ray analysis. Crystal data : A crystal of 3, size 0.3x0.4 x0.1 mm, was mounted on a Philips PW 1100 automatic diffractometer, equipped with a graphite monochromator and operating with CuK_α radiation (λ=1.5418 Å). The system is orthorhombic, space group $P2_12_12_1$ (Z=4) with cell parameters a=15.279 (4) ; b=25.418 (5) ; c=7.001 (3) Å.

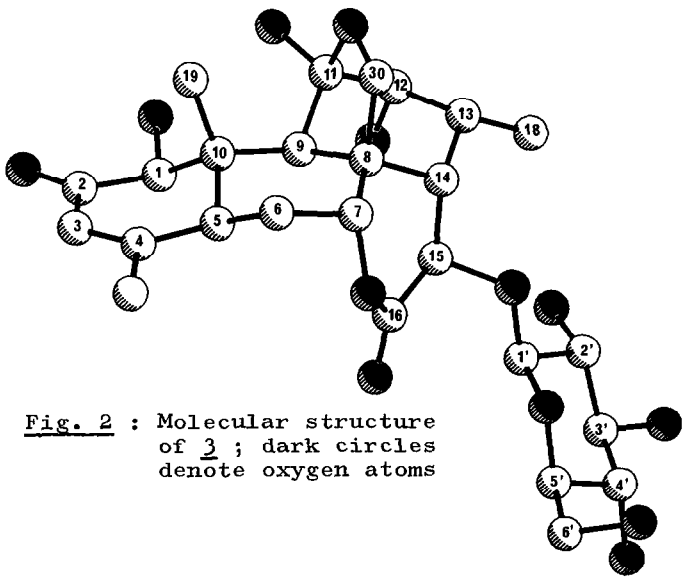


Fig. 2 : Molecular structure of 3 ; dark circles denote oxygen atoms

Over a total of 2688 reflections collected up to θ=60°, 2451 have been considered as observed above the 2σ background level. The structure has been sol-

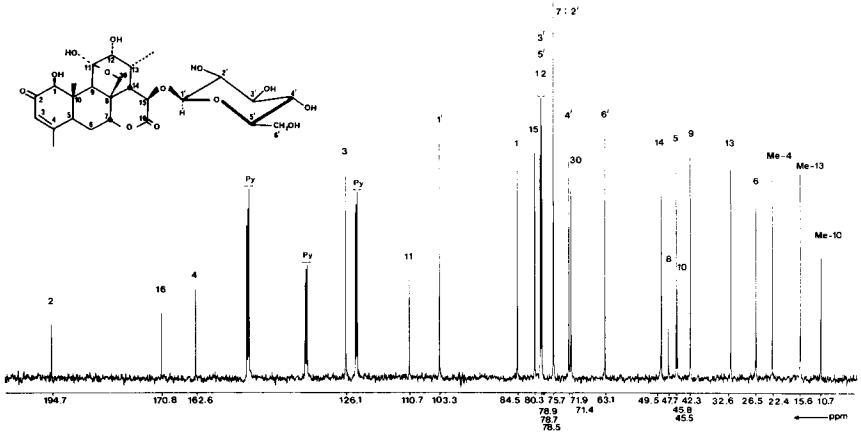
ved by using a Patterson Search program[11] with a starting model taken from the data of the p-bromobenzoate derivative of glaucarubin[5]. The refinements were carried out with isotropic thermal factors for the heavy atoms to $R=\Sigma||Fo|-|Fc||/\Sigma|Fo|=9.5\%$. The use of anisotropic thermal factors for the heavy atoms led to a final value of R=5.7%. The molecular view is given in Fig.2[12].

TABLE : 400 MHz ^{1}H-n.m.r. spectra of quassinoids 3 and 4 in pyridine-d_5 [δ in ppm, J as (Hz)]

	3	4		3	4
H-1	4.87 s	3.93 d (6.6)	H-30	3.80 (AB q, 9)	3.79 (AB q, 9)
H-2		4.58 m (6.6)		4.10	4.20
H-3	6.08 br. s	5.76 br. s	Me-4	1.72 s	1.66 s
H-5	3.2 d (12)	2.80 d (13.3)	Me-10	1.55 s	1.56 s
H-6e	2.15 dd (12, 2)	2.01 d (13.5)	Me-13	1.67 d (7)	1.65 d (7)
H-6a	1.97 dd (12, 2)	1.88 dd (13.5)	H-1'	5.46 d (7.5)	5.39 d (7.5)
H-7	4.28 br. s	4.77 br. s	H-5'	3.95 m	3.90 m
H-9	3.23 s	2.98 s	H-6'A	4.54 dd (12,3)	4.50 dd (12,3)
H-12	4.03 br. s	4.05 br. s	H-6'B H-2' H-3' H-4'	4.28 to	4.04
H-13	2.65 m	2.59 dd (6.7)			
H-14	2.67 dd (9,3)	2.65 m			
H-15	5.65 d (9)	5.59 d (10)			

Quassinoid 4 crystallized from methanol, m.p. 263-264°, $[\alpha]_D^{22}$+12.1 (c=0.99 ; pyridine). Its molecular formula was established by elemental analysis and FAB mass spectroscopy as $C_{26}H_{38}O_{13}$ (MH^+ at m/z 559) and differed from that of 3 by two hydrogen atoms. Compound 4 possesses like quassinoid 3 a hexose moiety as shown by the positive anthrone test and by the presence of an abundant fragmentation ion at m/z 379 (MH^+-180) in the FAB mass spectrum. The i.r. spectrum (nujol) showed hydroxyl absorption at 3350 cm^{-1} and only one carbonyl band at 1720 cm^{-1}. The absence of an u.v. absorption characteristic of an A-ring enone system and analysis of the 400 MHz ^{1}H-n.m.r. spectrum of 4 (Table) suggested that this quassinoid contains a vicinal glycol in ring A instead of an α-ketol, both types of compounds frequently co-occuring in the same plant[7]. Acetylation (acetic anhydride - pyridine) of quassinoid 4 afforded an octaacetate $C_{42}H_{54}O_{21}$. MS : (M^+-60) at m/z 834 and base peak at m/z 331 corresponding to the tetraacetyl hexose oxonium ion ;

Fig. 1 : 100.6 MHz ^{13}C (proton broad band decoupled) n.m.r. spectrum of 3 in pyridine-d_5



400 MHz ^{1}H-n.m.r. ($CDCl_3$) : three proton signals at δ 1.00 (d, J=7,5, Me-13), 1.40 (s, Me-10), 1.70 (s, Me-4) and eight resonances due to the acetoxy groups at δ 1.81, 1.97, 1.99, 2.00, 2.02, 2.11, 2.14 and 2.18.

Thus, the aglycone of 4 proves to be glaucarubol 6 and the hexose moiety is assumed to be located at C-15. Accordingly, on MnO_2 oxidation of 4 some quassinoid 3 was detected by tlc in the reaction mixture.

In the murine P-388 lymphocytic leukemia test system[13], glucosides 3 and 4, which are moderately water soluble, were found to be toxic down to the level of 2 mg/kg.

While esters of quassinoids are rather common, quassinoid glucosides are rarely found and only the following five have been isolated to date : bruceosides A and B, bruceantosides A and B[15], and picrasinoside A[16].

Acknowledgements : We are gratiful to Mr. Severin and Mr Lognay (Faculté Agronomique de Gembloux, Belgique) for providing us with defatted seeds of S. glauca. We thank Mme C. Fontaine and Mr J.-C. Beloeil for ^{1}H and ^{13}C n.m.r. measurements and Mr P. Varenne for FAB mass spectra. This investigation was supported by PHS Grant number 2 R01 CA 26699-03 awarded by the National Cancer Institute, DHHS.

REFERENCES AND NOTES

1. For previous paper in this series see Nguyen-Ngoc-Suong, S. Bhatnagar, J. Polonsky, W. Vuilhorgne, T. Prangé and C. Pascard, Tetrahedron Letters 23, 5159 (1982).
2. A.J. Caruso, J. Polonsky and B.S. Rodriguez, Tetrahedron Letters, 23, 2567 (1982).
3. E.A. Ham, H.M. Schafer, R.G. Denkewalter and N.G. Brink, J. Am. Chem. Soc., 76, 6066 (1954).
4. J. Polonsky, Cl. Fouquey and A. Gaudemer, Bull. Soc. Chim. Fr., 1818, 1827 (1964).
5. G. Kartha and D.J. Haas, J. Am. Chem. Soc., 86, 3630 (1964).
6. A. Gaudemer and J. Polonsky, Phytochemistry, 4, 149 (1965).
7. J. Polonsky, Fortschr. Chem. Org. Naturst., 30, 101 (1973).
8. Quantitative HPLC analysis of these quassinoids has recently been described : X. Monseur and J.C. Motte, J. of Chromatogr., 264, 469 (1983).
9. J. Polonsky, Z. Baskevitch, H.E. Gottlieb, E.W. Hagaman and E. Wenkert, J. Org. Chem., 40, 2499 (1975).
10. C. Le Cocq and J.Y. Lallemand, J.C.S. Chem. Comm., 150 (1981).
11. B.P. Braun, J. Hornstra and J.L. Leenhouts, Philips Research Report, 24, 85 (1969).
12. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW.
13. Courtesy of Dr. M. Suffness (N.I.H., USA).
14. K.H. Lee, Y. Imakura, Y. Sumida, R.Y. Wu, I.H. Hall and H.C. Huang, J. Org. Chem., 44, 2180 (1979).
15. M. Okano, K.H. Lee, I.H. Hall and F.E. Boettner, J. Nat. Prod. (Lloydia) 44, 470 (1981).
16. M. Okano, N. Fukamiya, K. Kondo, T. Fujita and T. Aratani, Chem. Letters, 1425 (1982).

(Received in France 7 November 1983)