

STRUCTURE OF LAURYCOLACTONE A AND B , NEW C₁₈ - QUASSINOIDS FROM
EURYCOMA LONGIFOLIA AND REVISED STRUCTURE OF EURYCOMALACTONE
(X - RAY ANALYSIS)¹

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Summary · Lauricolactone A 2 and B 3 are new quassinoids with a C₁₈ basic skeleton isolated from the Vietnamese Simaroubaceae , *Eurycoma longifolia* Jack . The structure of Eurycomalactone, a C₁₉ quassinoid isolated previously from the same plant, has been revised and shown to be 4 . The structures have been established by spectral means and those of 2 and 4 confirmed by X-ray diffraction analysis.

Previous studies² of the chemical constituents of *Eurycoma longifolia* Jack, a Simaroubaceae common in Viet Nam, led to the isolation of the C₁₉ quassinoid, eurycomalactone, to which structure 1 has been attributed on the basis of chemical transformations. Later on³ ¹H- and ¹³C-NMR spectra were claimed to be consistent with the assigned structure 1 . However the arguments presented in the latter study³ do not lead to an unambiguous proof of structure 1 .

Further investigation of the plant extract has now led to the isolation and structural elucidation of two new C₁₈ quassinoids, named lauricolactone A 2 and B 3 . We also report the revision of structure 1 for eurycomalactone to structure 4 .

Lauricolactone A 2 m.p. 265-270° (decomp.) , $[\alpha]_D^{22} + 216^\circ$ (c=0.44, CHCl₃). The molecular formula C₁₈H₂₂O₅ was established by high resolution mass spectrometry with M⁺ at m/z 318.466 and abundant fragmentation ions at m/z 274.1575 (C₁₇H₂₂O₃ , M⁺-CO₂), 259.1339 (C₁₆H₁₉O₃ M⁺⁺ - CO₂ - CH₃) and the base peak at m/z 123.0809 (C₈H₁₁O) assigned to the fragment ion 5 . This ion probably arises by cleavage of the C₉, C₁₀ (Mc Lafferty rearrangement) and C₆, C₇ carbon bonds , a peak of low intensity at m/z 109.0647 (C₇H₉O) was also present . The i r spectrum (CHCl₃) showed carbonyl bands at 1775 (γ-lactone), 1715 (ketone) and 1690 cm⁻¹ (α,β -unsaturated ketone), and , in agreement with the formulation of ring A as in 2 , the u.v spectrum showed a maximum at 230 nm (ε=9.000) . The 400 MHz ¹H-NMR spectrum (Table I) of lauricolactone 2 was particularly revealing and with extensive decoupling experiments allowed the identification of all proton resonances. The presence of the hydroxyl group at C-11 was suggested by the appearance of a signal at δ 5.00 as a doublet of doublets . Furthermore, the spectrum showed that H-13 is only slightly coupled with H-14 and H-12 . These negligible couplings as well as the observed long range coupling between H-12 and H-14 suggest the predominance of a similar conformation in solution to that observed in the solid state (vide infra) . Inspection of the ¹³C-NMR spectrum (Table II) of

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Table I 400 MHz ^1H NMR spectral data for 2^a, 3^b and 4^b (δ in ppm)

	<u>2</u>	<u>3</u>	<u>4</u>
H-1	-	-	4.05 br s ^c
H-2	5.85 q	6.13 br d	-
H-3	-	-	6.11 br s
H-5	2.70 br dd	-	2.80 m ^e
H-6e	2.80 q	5.91 s	2.65 dd
H-6a	2.10 q	-	2.75 dd
H-9	1.95 d	2.13 d	1.86 d
H-11	5.00 br dd	5.03 br dd ^d	4.78 br dd ^d
H-12	4.30 dd	4.30 dd	4.38 br d
H-13	3.06 br q	3.03 m	2.8 m ^e
H-14	2.77 d	2.90 d	2.88 d
Me-4	2.1 br s	2.21 d	1.95 br s
Me-8	1.46 s	1.6 s	1.56 s
Me-10	1.52 s	1.7 s	1.26 s
Me-13	1.14 d	1.16 d	1.16 d
OH-1	-	-	4.5 d
OH-11	-	2.58 d	3.16 d

Coupling constants in Hz

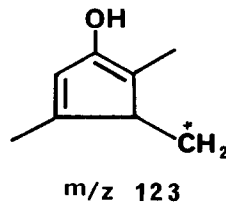
<u>2</u>	$J_{2,\text{Me-4}}=0.5$, $J_{6a,6e}=16$, $J_{6e,5}=13$, $J_{6a,5}=5$ $J_{9,11}=3.5$, $J_{11,12}=5$, $J_{12,13}=\text{ca } 0$ $J_{12,14}=1$, $J_{14,13}^{\text{ca } 1}$, $J_{13,\text{Me-13}}=7$
<u>3</u>	$J_{2,\text{Me-4}}=1.25$, $J_{9,11}=3.5$, $J_{11,12}=5$, $J_{12,13}=\text{ca } 0$, $J_{12,14}=1$, $J_{13,\text{Me-13}}=7$, $J_{11,\text{OH}}=5.6$
<u>4</u>	$J_{3,5} \leq 1$, $J_{3,\text{Me-4}} \leq 1$, $J_{6e,6a}=15.5$, $J_{6e,5}=4$, $J_{6a,5}=13.5$, $J_{9,11}=3.5$, $J_{11,12}=5$, $J_{12,13}=\text{ca } 0$, $J_{12,14}=1$, $J_{13,\text{Me-13}}=7$, $J_{1,\text{OH}}=1.5$, $J_{11,\text{OH}}=5.8$

- a In $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{OD}$, b in CDCl_3
 c In CD_3OD appears as a sharp singlet,
 d J-Values calculated from spectra recorded in CD_3OD
 e Complex pattern of overlapping signals

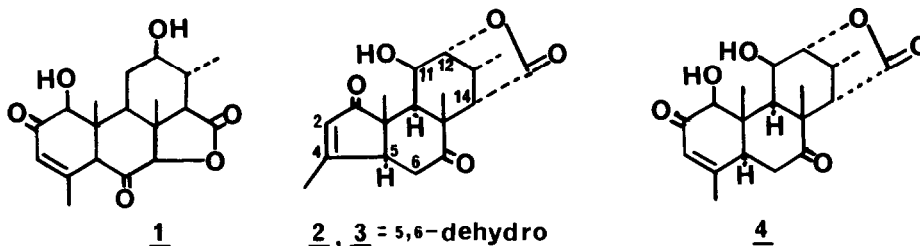
Table II 100.6 MHz ^{13}C NMR spectral data for

	<u>2</u> ^a	<u>3</u> ^b and <u>4</u> ^b
C-1	214.4 s	205.4 81.3
C-2	127.2 d	133.0 197.4
C-3	-	- 124.5
C-4	178.5 ^c s	166.4 ^c 162.2
C-5	53.7 d	165.0 ^c 49.5 ^c
C-6	41.8 t	116.3 36.3
C-7	210.4 s	198.2 205.5
C-8	49.0 ^d s	45.5 47.0
C-9	51.7 d	53.2 53.0
C-10	47.7 ^d s	52.2 51.2
C-11	66.7 d	67.8 69.9
C-12	84.3 d	83.6 83.2
C-13	31.3 d	32.2 32.4
C-14	36.6 d	40.7 49.2 ^c
C-15	178.0 ^c s	176.5 176.2
Me-13	16.3 ^e q	16.9 16.7
Me-10	17.4 ^e q	13.9 12.2
Me-4	20.9 q	21.5 22.0
Me-8	24.4 q	23.1 23.7

The ^{13}C multiplicities for 2 were determined by off-resonance decoupling and also by modulated spin-echo technique⁴ and those for 4 by the latter technique. The chemical shift assignments for 3 were made by analogy with those for 2 and 4
 a In $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{OD}$,
 b In CDCl_3
 c-e Values may be interchanged within any vertical column



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laurycolactone A provides further confirmation for the proposed structure 2. Unequivocal proof for this structure was provided by single-crystal X-Ray analysis using crystals of 2 obtained from ethanol. The molecular structure of 2 is shown in Figure 1.

Chromatography of the mother liquors of laurycolactone A 2 yielded a small amount of laurycolactone B 3 which crystallized from ethanol as large prisms, m p 228–230°, $[\alpha]_D^{22} + 92.6^\circ$ (c=0.364, CHCl_3), $\text{C}_{18}\text{H}_{20}\text{O}_5$ ($M^+ = 316$). Its i.r. spectrum showed hydroxyl absorption at 3400 cm^{-1} and carbonyl bands at 1775 (γ -lactone), 1700 and 1665 cm^{-1} (cross-conjugated dienedione)⁵. The u.v. spectrum displayed an absorption maximum at 285 nm ($\epsilon = 13,904$) supporting the presence of a cross-conjugated dienedione chromophore. The 400 MHz $^1\text{H-NMR}$ spectrum (Table I) of laury-

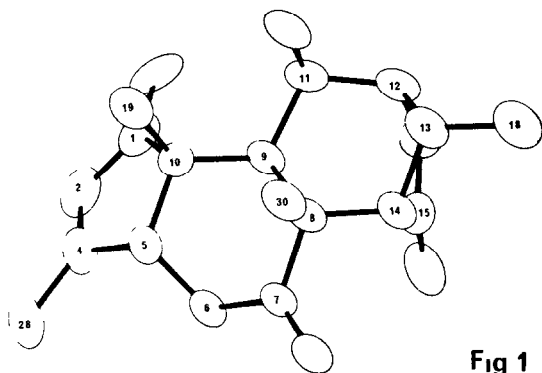


Fig 1

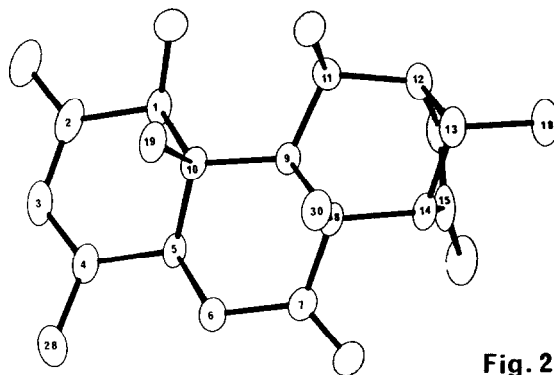


Fig. 2

colactone B was in good agreement with the proposed structure 3. It showed nearly identical chemical shifts and multiplicities of the hydrogen atoms located in ring C, revealed an additional olefinic proton signal at $\delta 5.91$ and lacked the methylene protons at C-6. The $^{13}\text{C-NMR}$ spectrum (Table II) of laurycolactone B fully confirmed its structure 3 and showed, as expected, besides the three carbonyl resonances, four additional sp^2 carbon atoms ($\delta 166.4, 165.0, 133.0$ and 116.3).

Laurycolactone A 2 and B 3 are closely related to samaderine A, isolated from *Samadera indica*⁵, which was the first C_{18} -quassinoid to have its structure determined. These quassinoids with a contracted A-ring as well as the known C_{19} -quassinoids (the various samaderins and cedronins)⁶ have the 12-hydroxyl involved in their γ -lactone ring. This fact prompted us to reinvestigate the previously proposed structure¹ of eurycomalactone. A sample of eurycomalactone isolated by one of us (N.N.S.) was purified by repeated preparative t.l.c. (AcOEt - hexane, 1:1) to give pure eurycomalactone, $\text{C}_{19}\text{H}_{24}\text{O}_6$, ($M^+ = 348$), $\lambda_{\text{max}}^{\text{EtOH}}$ 242 nm ($\epsilon = 14774$), $[\alpha]_D^{22} + 104.2^\circ$ (c=0.144, CHCl_3). The 400 MHz $^1\text{H-NMR}$ spectrum (Table I) of eurycomalactone was very informative and was consistent with structure 4 but not with 1. Comparison of this spectrum with that of laurycolactone A 2 indicated that the structural difference between

these quassinoids resided only in the A-ring, Thus, H-11 is assigned the signal (dd) at δ 78, H-13 is only weakly coupled with H-14 and H-12 and a long range coupling is observed between these two protons (*vide infra*). Further evidence for the revised structure 4 for eurycomalactone was obtained by interpretation of its ^{13}C -NMR spectrum (Table II) For a complete structural analysis, however, a single crystal X-ray analysis was carried out using crystals of eurycomalactone 4 obtained from methanol solution. The molecular structure of 4 is shown in Figure 2. Laurycolactone A 2 did not display significant inhibition of cell transformation induced by Rous sarcoma virus⁷ nor against the P-388 lymphocytic leukemia

Crystal data Crystals of laurycolactone A 2 belong to the orthorhombic space group $P2_1^2 2_1^2$ (Z=4) with the cell parameters of $a = 13.774$, $b = 11.926$ and $c = 9.721$ Å and those of eurycomalactone 4 belong to the monoclinic space group $P2_1$ (Z=2) with $a = 13.561$, $b = 6.263$, $c = 10.451$ Å and $\beta = 107.5^\circ$. The data have been recorded on a Philips PW1100 Four Circle diffractometer using the $\text{CuK}\alpha$ radiation ($\lambda = 1.5418$ Å) monochromatized by graphite. 1114 and 1379 structural factors above the 2σ background level have been derived from the measured intensities, respectively. Both structures have been solved by direct methods. All the atoms of eurycomalactone 4 have been found in the E-map corresponding to the highest figure of merit and the structure of laurycolactone A 2 was obtained after a straightforward application of MULTAN program and the use of six selected symbolic phases in the starting set. Both structures have been anisotropically refined to a R index of 5.7 and 6.3%, respectively. All hydrogen atoms were found on difference Fourier syntheses and introduced in the subsequent computations with an isotropic factor equal to that of the bonded carbon, they were not refined. The figures 1 and 2 display the ORTEP representation of these two compounds viewed approximately perpendicular to the B/C ring system⁸. The dihedral angles between H-12 and H-13, and between H-13 and H-14 of laurycolactone A 2 are 82.5° and 67° , respectively, those for eurycomalactone 4 are 79° and 104° , respectively. These values account for the observed negligible J^3 couplings of these protons. Moreover, the long range coupling between H-12 and H-14 is well explained by their planarity. In both structures, the four atoms H-12, C-12, C-14 and H-14 are nearly coplanar, with a maximum deviation lower than 0.05 Å out of their mean plane; the C-13 atom of the W conformation lies 0.4 Å for 2 and 0.7 Å for 4 above that plane.

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REFERENCES AND NOTES

- 1- For previous paper in this series see A. J. Caruso, J. Polonsky and B. S. Rodriguez, Tetrahedron Letters, **23**, 2567 (1982)
- 2- Le-Van-Thoi and Nguyen-Ngoc-Suong, J. Org. Chem., **35**, 1104, (1970), and references therein
- 3- A. Oei-Koch and L. J. Kraus, Sci. Pharm., **48**, 110 (1980)
- 4- C. Le Cocq and J. V. Lallemand, J. C. S. Chem. Comm., 150 (1981)
- 5- J. Polonsky, J. Zylber and R. O. B. Wijesekera, Bull. Soc. Chim. France, 1715, (1962), M. C. Wani, H. L. Taylor, M. E. Wall, A. T. McPhail and K. D. Onan, J. C. S. Chem. Comm., 295 (1977)
- 6- J. Polonsky, Fortschr. Chem. Org. Naturst., **30**, 101 (1973)
- 7- Pierré, A., Robert-Gero, M., Tempête, C., Polonsky, J., Biochem. Biophys. Res. Commun., **93**, 675 (1980)
- 8- 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 AEW

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