X-ray Crystal Structure of Lygodinolide: A Novel Spiro Furopyran–Perhydrophenanthrene Derivative from *Lygodium flexuosum*

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In a previous paper,[1] we reported the structure elucidation of *O-p*-coumaryl dryocrassol, a new compound isolated along with the known compounds tectoquinone, kaempferol, kaempferol-3-β-D-glucoside, β-sitosterol, stigmasterol, and dryocrassol itself from *Lygodium flexuosum* (Polypodiaceae), a fern widely distributed in India and known to possess potent antifertility activity.[2] Further investigation on the methanolic extract of this plant afforded, in 0.002% yield, yet another new compound, lygodinolide (1), the characterization of which is described herein.

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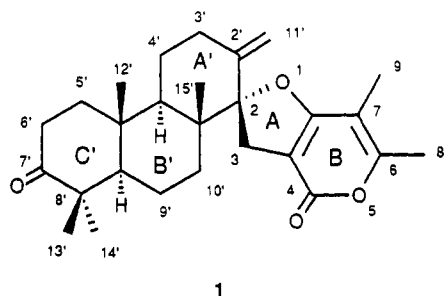
Lygodinolide ($C_{27}H_{36}O_4$; M^+ m/z 424; mp 278 °C; $[\alpha]^{28}_D$ +42.0° (c 0.1, $CHCl_3$)) was crystallized from $CHCl_3$–petroleum ether. Its IR spectrum showed two peaks in the carbonyl region at 1710 and 1695 cm^{-1}, the former being more intense than the latter; another sharp peak at 890 cm^{-1} was assignable to a >C=CH$_2$ group, the presence of which was supported by the ^{1}H NMR spectrum (2 H, s, at δ 5.0), which also contained singlets for four methyl groups. Two other methyl group signals were somewhat downfield, but the existence of either an acetoxy or a ketomethyl group in the molecule could be discounted since 1 was unaffected by mild alkaline hydrolysis and NaBH$_4$ only reduced a ring ketone to a secondary alcoholic function (1 H, dd, $J = 11$, 5 Hz at δ 3.24). The ^{1}H NMR spectrum of 1 additionally displayed a two-hydrogen AB doublet of doublets ($J = 16$ Hz) at δ 3.05, compatible with a methylene group flanked by fully substituted carbon atoms. In the mass spectrum, a prominent fragment ion peak at m/z 205 was reminiscent of the A/B ring fragment of a 3-keto triterpenoid. The presence of such a moiety was indeed substantiated by the virtually identical ^{13}C NMR signals observed (compared to methyl oleanonate) for the corresponding carbon atoms of 1 except for C$_8$ and C$_9$ (C$_{10a'}$ and C$_{4a'}$, respectively, in 1) which were shifted by about 4 δ units, reflecting the different nature of the C ring (A' in 1). The spectrum, which displayed separate peaks for all 27 carbon atoms, contained two low-field signals at
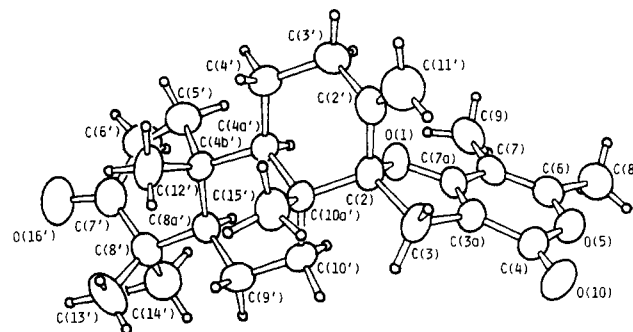
Figure 1. ORTEP diagram showing the structure and solid-state conformation of lygodinolide (1). Small circles represent hydrogen atoms.

δ 110.6 and 144.7 consistent with an *exo*-methylene group. Other peaks in the downfield region (singlets at δ 98.8, 100.7, 103.0, 160.1, 161.8, and 169.4) pointed to an appropriately substituted aromatic or conjugated chromophore.

Single-crystal X-ray analysis established unequivocally the complete structure and relative stereochemistry of 1.[3] A view of the structure is presented in Figure 1. Bond lengths and angles are in accord with expected values.[4] Ring A has an envelope conformation, ring B is fairly planar, and cyclohexane rings A' and B' are in chair forms, while ring C' approximates to a twist-boat conformation.

Spectral data for 1 are consistent with the derived structure. The UV spectrum resembles that of helipyrone (2).[5] In the IR spectrum, the 1710-cm^{-1} absorption arises

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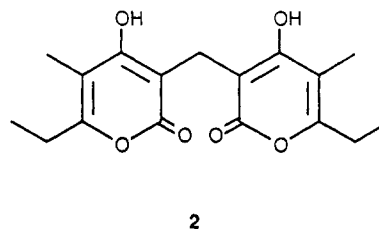
only partly from the keto carbonyl stretching frequency as evidenced by the fact that the NaBH$_4$ reduction product of 1 showed two carbonyl peaks of comparable intensity at 1710 and 1695 cm^{-1}. Literature survey[6] reveals that most unsaturated lactones show a split $\nu_{C=O}$ in this region, believed to arise due to Fermi resonance with an overtone. Two other absorption bands at 1635 and 1565 cm^{-1} are in agreement with ν_{ring} values reported for 4-oxygenated α-pyrones. Of the ^{13}C NMR signals, those for C$_{4a'}$–C$_{10a'}$ along with substituents could, as above for the mass spectral data, be assigned by comparison with like 3-oxo triterpenoids. Further, those for C$_{2'}$–C$_{4'}$ were derived from chemical shifts[7] of the B-ring carbon atoms of some labdane diterpenes, while most of the assignments for the pyrone moiety could be made on the basis of confirmed values[5] for 2. The chemical shifts attributed to the methyl groups at C$_6$ and C$_3$ rely, on the other hand, on the observed higher J_{res} values in the SFORD spectrum. In

(3) Supplementary material. See the paragraph at the end of the paper.
(4) Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. *J. Chem. Soc., Perkin Trans. 2*, 1987, S2.
(5) Ali, E.; Bagchi, D.; Pakrashi, S. C. *Phytochemistry* 1982, *21*, 243.
(6) Katritzky, A. R.; Ambler, A. P. In *Physical Methods in Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Academic Press: New York, 1963; Vol. II, p 165.
(7) Chang, C. W. J.; Flament, I.; Matson, J. A.; Nishida, T.; Ohloff, G.; Wehrli, F. W.; Weinheimer, A. J. In *Progress in the Chemistry of Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer-Verlag: New York, 1979; Vol. 36, p 1.

(1) Achari, B.; Basu, K.; Saha, C. R.; Pakrashi, S. C. *Planta Med.* 1986, 52, 329.
(2) Gaitonde, B. B.; Mahajan, R. T. *Indian J. Med. Res.* 1980, 72, 597.

absence of a suitable model, however, the singlet at δ 98.8 has been assigned to the spiro carbon center by elimination, although the chemical shift is somewhat high for an sp^3-carbon carrying one oxygen atom (the downfield shift possibly stems from the β-substitution pattern coupled with the conjugation of the oxygen atom with the enone moiety). Nevertheless, some of the close-lying resonances could be exchangeable.

Peaks at m/z 205 and 219 in the mass spectrum of 1 conceivably arise from an initial breaking of the C_2–$C_{10a'}$ bond followed by C_3–$C_{4'}$ or $C_{4'}$–$C_{4a'}$ cleavage with hydrogen shift as appropriate and thus could originate from either part of the molecule. Another pair of peaks at m/z 271 and 153 plausibly arise from the two fragments derived via the rupture of O_1–C_2 and C_2–C_3 bonds with one hydrogen atom transfer. There is also a significant peak at m/z 326, genesis of which may be traced in the cleavage of ring C′ with elimination of C_5–$C_{8'}$, although this is not a very prominent occurrence in triterpenoid ketones.

The spiro furopyran–perhydrophenanthrene skeletal structure of 1 appears to represent a new class of natural product for which the biogenesis is not readily discernible. Biogenetically, 1 may be derived from diterpenoid and pyran parts. Since cheilanthane-type sesterterpenoids have been reported from Polypodiaceae, however, derivation of 1 from such a precursor (3) through biogenetic incorporation of a two-carbon unit (or two one-carbon units) with methyl 1,2-transfer may not be ruled out.

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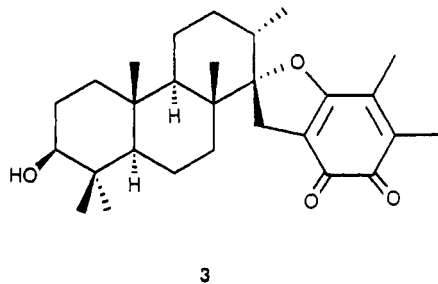
Experimental Section

The melting point was determined in an open capillary and is uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 100 and 25 MHz, respectively, using $CDCl_3$ as solvent and TMS as internal standard. The mass spectrum was recorded at 70 eV with a direct inlet system.

Plant Material. Whole plant of *L. flexuosum* was collected by M/s United Chemicals and Allied Products, Calcutta, where a voucher specimen is maintained.

Extraction and Isolation. The air-dried whole plant (4 kg) was percolated with MeOH for 90 h at room temperature. Distillation of the methanolic percolate on a water bath was followed by extraction of the residue with ether (3 × 500 mL). The ether was then removed on a water bath, leaving 60 g of crude extract, which was column chromatographed over silica gel (60–120 mesh). The 25% chloroform in petroleum ether eluted fraction when rechromatographed over neutral alumina afforded, in 5% chloroform in petroleum ether eluants, lygodinolide (1): 82 mg, 0.002%; mp 278 °C (CHCl$_3$–petroleum ether); $[\alpha]^{28}_D$ +42.0° (c 0.10, CHCl$_3$); UV λ_{max} MeOH (log ϵ) 282 sh (3.27), 251 sh (3.43), 222 sh (3.86), 211 sh (3.95) nm; IR ν_{max} (Nujol) 3600–3100, 2900,

1710, 1695, 1635, 1575, 1430, 1380, 1270, 1120, 1050 and 890 cm^{-1}; ^{1}H NMR δ 5.0 (s, 2 H, CH$_2$), 3.05 (2 d, 2 H, J = 16 Hz, C$_3$-CH$_2$), 2.64–2.36 (m, 12 H), 2.20 (s, 3 H, C$_6$-CH$_3$), 1.95 (s, 3 H, C$_7$-CH$_3$), 1.80–1.28 (m, 12 H); ^{13}C NMR δ 216.8 (s, C$_7'$), 169.4 (s, C$_4$), 161.8 (s, C$_{7a}$), 160.1 (s, C$_6$), 144.7 (s, C$_{2'}$), 110.6 (t, C$_{11'}$), 103.0 (s, C$_7$), 100.7 (s, C$_{3a}$), 98.8 (s, C$_2$), 54.6 (d, C$_{8a'}$), 50.7 (d, C$_{4a'}$), 47.0 (s, C$_{8'}$), 42.9 (s, C$_{10a'}$), 39.1 (t, C$_{5'}$), 36.9 (s, C$_{4b'}$), 33.7 (t, C$_{6'}$), 32.6 (t, C$_{10'}$), 32.4 (t, C$_{3'}$), 26.6 (t, C$_3$), 26.3 (q, C$_{14'}$), 22.1 (t, C$_{4'}$), 20.8 (q, C$_{13'}$), 19.1 (t, C$_{9'}$), 16.9 (q, C$_8$), 16.2 (q, C$_{15'}$), 15.9 (q, C$_{12'}$), 9.4 (q, C$_9$); MS, m/z 426, 409, 396, 381, 326, 311, 283, 271, 219, 205, 153.

Sodium Borohydride Reduction of 1. Compound 1 (5 mg) was added to excess NaBH$_4$ in CH$_3$OH. The reaction mixture was kept on a water bath for 3 h and then worked up in the usual way: IR ν_{max} (Nujol) 3600–3100, 1710, 1690, 1565, 1435, 1375, 1265, 1110, 1025, 890 cm^{-1}; ^{1}H NMR δ 5.0 (s, 2 H, CH$_2$), 3.24 (dd, 1 H, J = 11, 5 Hz, C$_7$-H), 3.05 (2 d, 2 H, J = 16 Hz, C$_3$-CH$_2$), 2.64–2.36 (m, 12 H), 2.20 (s, 3 H, C$_6$-CH$_3$), 1.95 (s, 3 H, C$_7$-CH$_3$), 1.80–1.28 (m, 12H).

X-ray Crystal Structure Analysis of Lygodinolide. Crystal data: C$_{27}$H$_{36}$O$_4$, M_r 424.59, orthorhombic, a = 11.146 (1) Å, b = 20.524 (2) Å, c = 10.199 (1) Å (from 25 orientation reflections, 60° < θ < 71°), V = 2333.1 (7) Å^3, Z = 4, D_{calcd} = 1.209 g cm^{-3}, μ(Cu Kα) = 5.9 cm^{-1} (λ = 1.5418 Å); crystal dimensions 0.09 × 0.18 × 0.50 mm, space group $P2_12_12_1$ (D_2^4) uniquely derived from the systematic absences $h00$ when $h \neq 2n$, $0k0$ when $k \neq 2n$, and $00l$ when $l \neq 2n$.

Oscillation and Weissenberg photographs yielded preliminary unit cell parameters and space group information. One octant of intensity data was recorded on an Enraf-Nonius CAD-4 diffractometer [Cu Kα radiation, graphite monochromator; ω–2θ scans, scan width $(1.00 + 0.14 \tan \theta)°$]. From a total of 2712 nonequivalent measurements, those 2102 reflections with I > $3.0\sigma(I)$ were retained for the structure analysis, and the usual Lorentz and polarization corrections were applied.

The crystal structure was solved by direct methods. All non-hydrogen atoms were located in an E-map. Several rounds of full-matrix least-squares adjustment of atomic positional and thermal parameters (at first isotropic and then anisotropic) were followed by evaluation of a difference Fourier synthesis that yielded hydrogen atom positions. Inclusion of hydrogen atom positional and isotropic thermal parameters, as well as an extinction correction, as variables in the subsequent least-squares iterations led to convergence (maximum shift 0.01σ) at R = 0.036 (R_w = 0.50, GOF = 1.2).[8]

Neutral atom scattering factors used in the structure factor calculations were taken from ref 9. In the least-squares iterations, $\sum w\Delta^2$ (w = $1/\sigma^2(|F_o|)$, Δ = $(|F_o| - |F_c|)$) was minimized. Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius SDP suite of programs incorporating the direct-methods program MULTAN11/82.

Supplementary Material Available: Tables of crystallographic data, fractional atomic coordinates, thermal parameters, bond lengths, bond angles, and torsion angles for 1 (12 pages). Ordering information is given on any current masthead page.

(8) R = $\sum ||F_o| - |F_c||/\sum|F_o|$; R_w = $[\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$; GOF = $[\sum w\Delta^2/(N_{observns} - N_{param})]^{1/2}$.
(9) *International Tables for X-ray Crystallography*; Kynoch: Birmingham, England, 1974; Vol. IV.