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THE ABSOLUTE CONFIGURATION OF COLEON A: A *SECO* DITERPENOID PIGMENT FROM *COLEUS* SPP.

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Key Word Index—*Coleus igniarius*; *C. kilimandjari*; Labiatae; 1,10 *seco* abietane; X-ray crystallography; ^{1}H NMR.

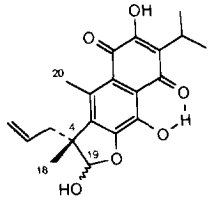
Abstract—The absolute configuration at C-4 of coleon A has been shown to be *R*. Coleon A crystallizes as a 1:1 mixture of (4*R*,19*R*) and (4*R*,19*S*) molecules. ^{1}H NMR studies of the solution configuration showed that the (4*R*,19*R*) form predominates in deuteroacetone.

INTRODUCTION

Coleon A (**1**) is an unique 1,10 *seco* abietane diterpenoid pigment which was originally isolated by Eugster *et al.* [1] from the leaves of *Coleus igniarius* Shweinf. The structure of the metabolite has been advanced on the basis of chemical and spectroscopic data [2], and its terpenoid origin has been confirmed from biosynthetic studies with [2-^{14}C] mevalonic acid [Ribi, M., Tullio, Di., Eugster, C. H. and Arigoni, D., unpublished results, cited by Eugster, C. H. in ref. 3. In the course of other work we have recently found that this metabolite is the principal leaf pigment of the related species *Coleus kilimandjari* Bally 9392 and have now determined the absolute configuration at C-4 of the metabolite by crystallographic methods and shown that it exists in two diastereomeric forms, both in the solid state and in acetone solutions.

RESULTS AND DISCUSSION

An ether extract of air dried leaves of *C. kilimandjari* was partitioned between toluene–pentane and aqueous methanol, the methanolic extract partitioned between brine and chloroform, and the chloroform extract subjected sequentially to adsorption chromatography on silica and partition chromatography on celite to afford **1**.



1

The material was identical (mixed mp, EIMS, IR, UV) with the metabolite isolated by Eugster *et al.* [1].

Solid state structures

The determination of absolute configuration was carried out by a method which we have recently described [3, 4]. The crystal structure of coleon A reveals that it crystallizes as a 1:1 mixture of two diastereomers both with the *R* configuration at C-4, and with opposite configurations [*S* in **1a**, *R* in **1b**] at C-19. In both molecules, the six-membered rings are essentially planar; the r.m.s. deviations of the atoms from their best plane in no case exceed 0.015 Å. The five membered rings have the envelope conformation, with atoms C (19a) and C (19b) 0.28 and 0.20 Å, respectively, out of the planes defined by the other atoms in the ring. Excluding the environment of C (4), the two molecules are related by an approximate inversion centre. This may be seen in Fig. 1, where the two molecules are shown, approximately in projection along [100]. Excluding atoms C(1)–C(3) and C(18) of both molecules, there is an inversion centre at the point [0.968 (19), 0.661 (28), 0.510 (13)].

The three hydroxyl protons are all involved in hydrogen bonding (Fig. 2). Each molecule has one internal hydrogen bond, O(7)–H(7)···O(14), and two pairs of external bonds, which hold the molecules together in ribbons parallel to [201]. It can be conjectured that this packing may be similar to the concentrated organization of the molecules in the dye glands of the plant [3].

Solution structures

Whereas the crystal structure of coleon A showed a 1:1 mixture of (4*R*,19*S*) and (4*R*,19*R*) molecules, ^{1}H NMR studies of the solution configurations in acetone-d_6 showed that one diastereomeric form (**1b**) predominates. In the

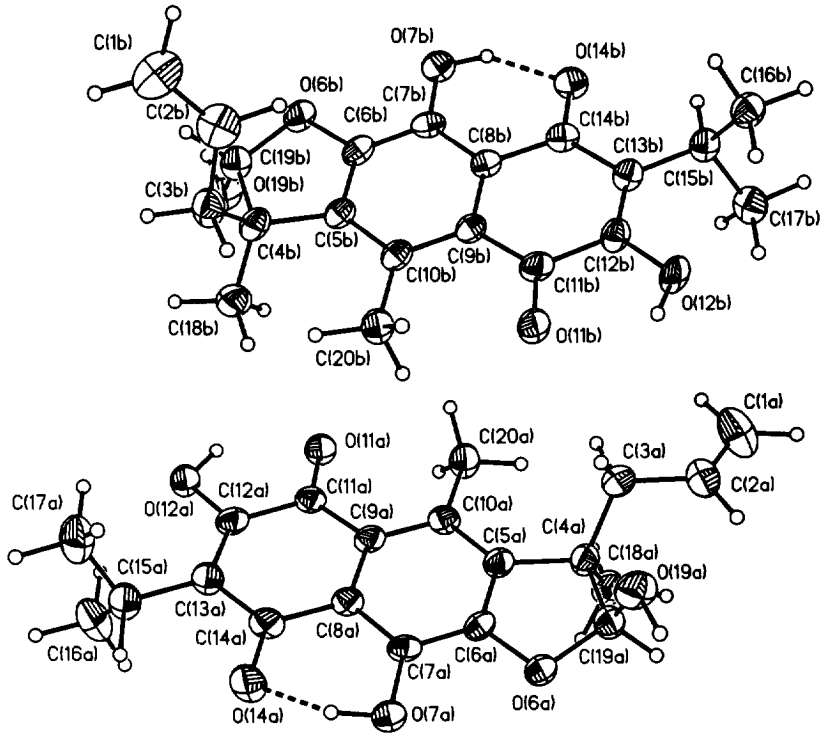


Fig. 1. View of two diastereomers of coleon A in the crystal structure. The upper molecule has the (4*R*,19*R*) configuration (**1b**) and the lower the (4*R*,19*S*) configuration (**1a**), the internal H-bond is shown.

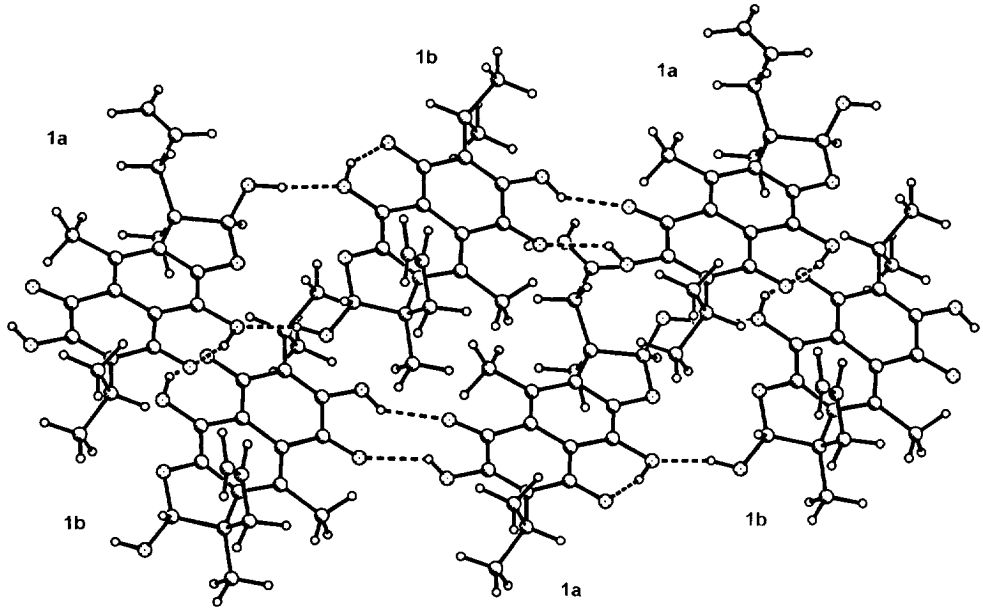


Fig. 2. Packing of coleon A molecules in the crystal structure showing the extended ribbon network.

solution spectra two resonances for the H-19 (methine), H-8 (methyl) and H-20 (methyl) were observed. Those of the major isomer (**1b**) at δ5.85 (H-19), 1.49 (H-18) and 2.65 ppm (H-20) and those of the minor isomer (**1a**) at δ5.73, 1.44 and 2.64 ppm, respectively. Assignment of the structures of the different isomers can be made on the basis of the observation of nuclear Overhauser effects (NOE) between these resonances (Fig. 3).

While both isomers show a predictable 1.5% NOE between their H-18 and H-20 singlet resonances (methyl groups at C-4 and C-10, respectively), only the minor diastereomer showed a significant NOE (2.5%) between

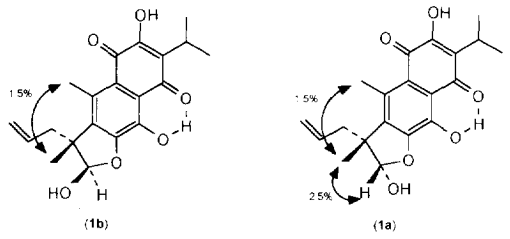


Fig. 3. Nuclear Overhauser effects observed for the two diastereomeric forms of coleon A in d_6-acetone solutions

the H-18 resonance and that of the methine proton at C-19, indicating that in the minor diastereomer the hydrogens of the C-4 methyl (H-18) were in close proximity to the lactol methine hydrogen (H-19). This is only possible if the stereochemistry of the C-19 centre is *S*. The ratio between the two diastereomeric forms is deuteroacetone solution is *ca* 3:1 on the basis of integration of the ^{1}H NMR signals.

EXPERIMENTAL

Metabolite isolation. Fresh leaves of *C. kilimandjari* (58 g) were dried at room temp. for 2 weeks, ground with a pestle and mortar, and the tissue extracted with Et_2O (500 cm^3) at room temp. overnight. The filtered ethereal extract was concd *in vacuo* and the residue partitioned between toluene–pentane (1/1, 50 cm^3) and 20% aq. MeOH (50 cm^3). The methanolic layer was evapd to dryness and partitioned between brine (20 cm^3) and $CHCl_3$ (50 cm^3). The dried $CHCl_3$ extract was evapd and subjected to CC on silica using a hexane–Et_2O gradient; frs containing coleon A were subjected to partition chromatography on celite using petrol (40–60°) as eluant and 20% aq. MeOH as the stationary phase to afford **1** (200 mg, 0.35% of fresh tissue wt) as red prisms from petrol [40–60° mp 137–139° (lit. [1] 136–136.5°)] $[\alpha]_D^{23}$ 100° (EtOH; *c* 1.0); EIMS *m/z* 358 $[M]^+$; IR $\lambda_{max}^{CCl_4}$ cm^{-1}; 3600, 3515, 3305, 2960, 1663, 1621: UV λ_{max} nm; 253 (17 000), 270 (12 000), 318 (10 800), 435 (6000): ^{1}H NMR (360 MHz acetone-d_6, major diastereomer): δ1.28 (6H, *d*, *J* = 7.1 Hz, H-16, H-17), 1.49 (3H, *s*, H-18), 2.65 (3H, *s*, H-20), 2.49 (2H, *m*, H-3), 3.33 (1H, *septet*, H-15), 5.05 and 5.60 (3H, *m*, H-1 and H-2), 5.85 (1H, *s*, H-19): resonances for protons at the 1, 2, 3, 15, 16 and 17 positions in the minor diastereomer overlapped those of the major diastereomer; the following signals could be individually assigned; δ1.44 (3H, *s*, H-18), 2.64 (3H, *s*, H-20), 5.73 (1H, *s*, H-19).

Crystal data. The metabolite crystallized as red triclinic crystals: $C_{20}H_{22}O_6$, *M* = 258.36, triclinic, space group *P*1 (no. 1), with *a* = 8.2274(12), *b* = 9.5382(14), *c* = 12.4307(18) Å, α = 103.515(8), β = 102.894(8), γ = 98.408(9)°, *V* = 904.1 Å^3 [from accurate 2θ values obtained at $\pm\omega$ for 36 reflections with $25 < 2\theta < 31°$, λ = 1.54184 Å, *Z* = 2, D_{calc} = 1.316 g cm^{-3}, *T* = 173.0 (2)K, red needle, 0.9 × 0.5 × 0.35 mm, μ = 0.76 mm^{-1}, *F*(000) = 380.

Data collection and processing. Carried out on a Stoe Stadi-4 4-circle diffractometer equipped with Oxford Cryosytems low temp. device [5], graphite monochromated Cu*K* X-radiation, *T* = 173 K, $\omega/2\theta$- scans with ω-scan width $(1.0 + 0.35\tan\theta)^0$, 4280 unique reflections were measured to $2\theta_{max}$ = 120°, including Friedel pairs for all data with $2\theta < 90°$, giving 4023 with $F > 4\sigma(F)$ for use in all calculations. No significant crystal decay or movement was apparent.

Structure solution and refinement. Automatic direct methods [6] located 39 atoms, which were expanded to the full 52 non-hydrogen atoms by subsequent difference Fourier syntheses. These atoms were then refined anisotropically [7]; hydrogen atoms bonded to carbon were inserted in calculated positions (C-H = 1.08 Å) with fixed temp. factors (U_{iso} = 0.05 Å^2); those bonded to O were found in difference maps and refined positionally. At final convergence *R*, R_w = 0.045, 0.065, respectively, *S* = 1.10 for 500 refined parameters and the final ΔF synthesis showed no peak or trough outwith $\pm$ 0.4 eÅ^{-3}. An extinction parameter converged to 1.3×10^{-2}. No absorption corrections were made. The weighting scheme $w^{-1} = \sigma^2(F)$ gave satisfactory agreement analyses; in the final cycle, $(\Delta/\sigma)_{max}$ was 0.18. Determination of absolute configuration was by a method previously described [4]: at convergence, for the 10 most discriminating Friedel pairs, the figure of merit ΣFoFc/Σ|FoFc| was 0.89. Inlaid [7] atomic scattering factors were used, molecular geometry calculations utilized CALC [8], and the Figs were produced by SHELXTL/PC [9].

The X-ray data have been deposited at the Cambridge Crystallographic Data Centre.

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