CONFORMATIONAL STUDY OF THE CEMBRANOID SARCOPHYTOL A, A POTENT ANTI-TUMOR-PROMOTER

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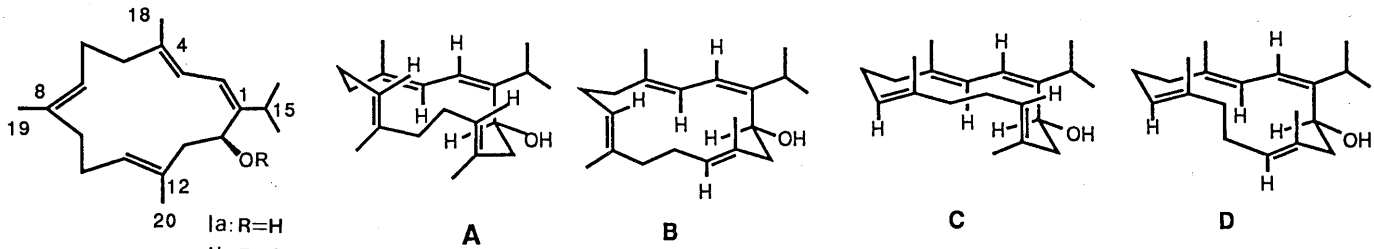
Conformational analysis of the marine cembranoid sarcophytol A (**1a**), a potent anti-tumor promoter, was carried out using a newly introduced molecular mechanic and molecular dynamic program Discover. Four minimum-energy conformations were derived, in accordance with a previous results of the epoxidation of **1a**, which afforded 7*R*,8*R*/7*S*,8*S*- and 11*R*,12*R*/11*S*,12*S*-epoxide pairs. The most stable conformation was the one having C-19 and C-20 directed opposite to C-18, with respect to the average plane of the fourteen-membered ring. X-Ray crystallography of sarcophytol A α-methoxy-α-trifluoromethylphenylacetate (**1c**) was carried out simultaneously. This confirmed the 14*S* absolute configuration of **1a** but the conformation of crystalline **1c** did not correspond to any of the four minimum-energy conformers of **1a**.

KEYWORDS cembranoid; sarcophytol A; conformation; anti-tumor promoter; Discover; molecular mechanics; X-ray analysis

Sarcophytol A (14*S*-monohydroxy-1*Z*,3*E*,7*E*,11*E*-cembratetraene, **1a**) is a simple cembranoid which has been isolated from *Sarcophyton glaucum*, a ubiquitous soft coral found in the coral reefs of Indo-Pacific coastal waters.[1)] Recently, Fujiki and co-workers found that **1a** is active as a potent anti-tumor-promoter in a two-stage carcinogenesis model (dimethylbenzanthracene-teleocidin) and also inhibits the hyperplasia of mouse dorsal skin; thus, the possibility exists that the compound inhibits chemical carcinogenesis.[2)] These effects were observed with an equimolar concentration of **1a** with respect to the tumor promoter teleocidin. It is an unparalleled potency when compared with the known natural anti-tumor promoters which are active only when a thousand times or more of the promoter quantity was applied.[1b)] It was suggested that **1a** binds specifically to the cytosolic fraction of mouse liver cells.[3)]

For future studies of the receptor-ligand interaction model, the conformational analysis of **1a** was undertaken. A molecular model of **1a** suggested that each plane, which involves 1*Z*,3*E*-diene or one of the two isolated double bonds, is not parallel but takes a heavily crossed disposition with respect to the average plane of the fourteen-membered ring. This is a characteristic of such medium-sized ring systems as germacrenes and cembrenes. Compound **1a** bears only one assymetric carbon, and the structure is composed simply by combination of three solid and nearly planar segments (C-14 and C-1 to C-5, C-6 to C-9, and C-10 to C-13) connected each other by single bonds. The ultraviolet (UV) absorption of **1a** indicated s-*trans* arrangement of the conjugated diene (252 nm, ε 20000).[1a)] This and the unusually large nuclear Overhauser effect (NOE) observed between H-3 and H-14 (15%)[1b)] show the coplanarity of the two double bonds of the conjugated diene. Thus, according to the

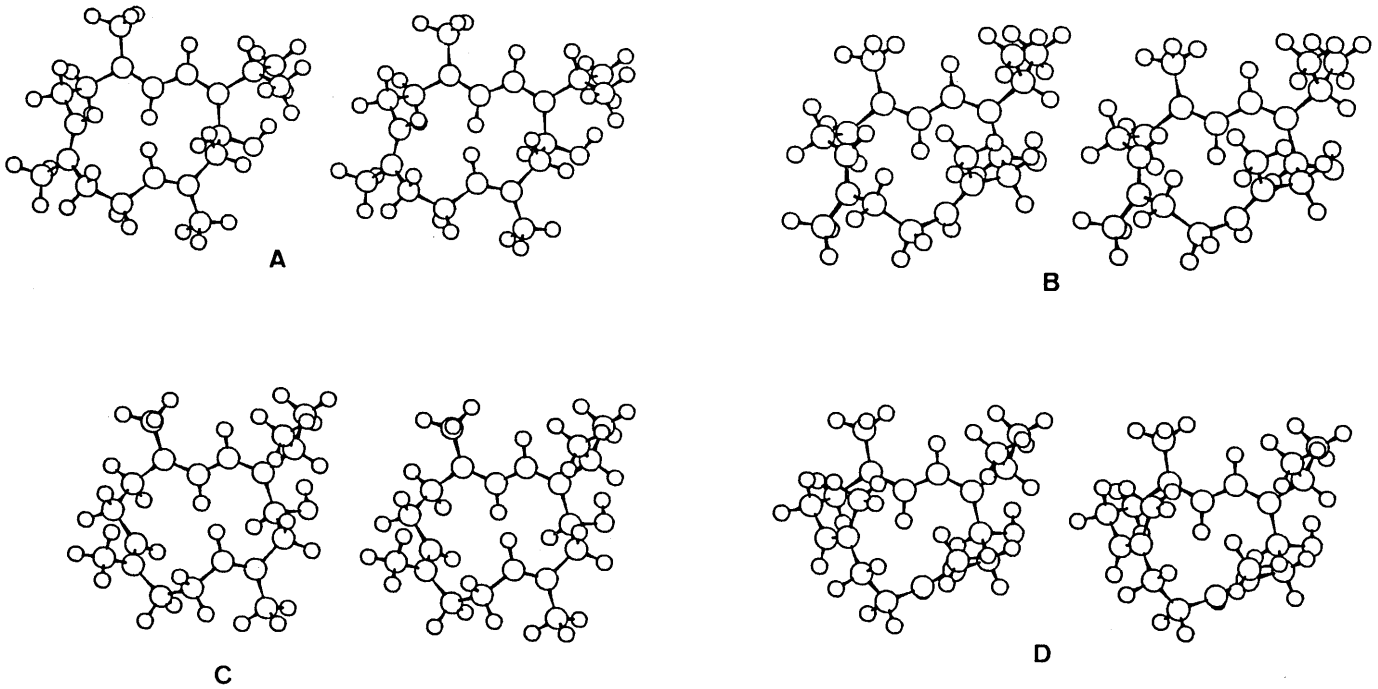


1a: R=H
1b: R=Ac
1c: R=MTPA

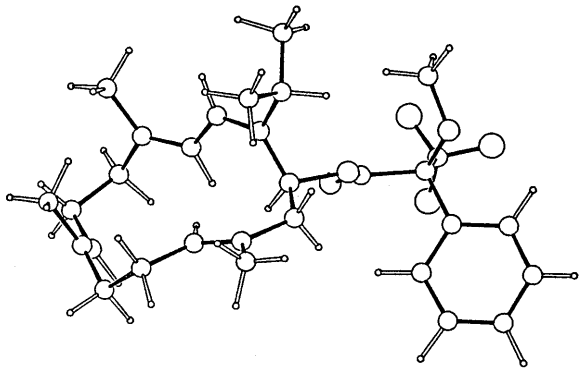
Fig. 1

direction of C-19 and C-20, with respect to the ring-plane, only four conformations would be possible [**A** (αα), **B** (αβ), **C** (βα) and **D** (ββ)][4,5] (Fig.1).

Based on these assumptions, the confomational analysis of **1a** was carried out by molecular mechanic calculation using the recently introduced molecular mechanic and molecular dynamic program Discover.[6] Discover is used generally for the conformational analyses of proteins and peptides, and is quite efficient for the analysis of such a simple compound as **1a**. Starting from the energy-minimized conformer **A**, energy minimization was carried out for the conformations derived by fixing the C-12,13 bond and rotating the C-8,9 bond over 0° to 180°. This process was repeated by rotating the C-12,13 bond every 10° over 0 to 180°. An approximate energy contour diagram of **1a** was obtained by this procedure, which showed the existence of four minimum energy conformers that corresponded to the confomers **A** to **D**. The energy minimizations based on the conformers **A** to **D**, with the maximum derivatives less than 0.01, gave the results shown in the perspective drawings below. The order of the total energies[7] are **A** (αα,-46.2 kcal), **C** (βα,-44.7 kcal), **B** (αβ,-43.4 kcal) and **D** (ββ,-41.4 kcal). Conformer **A** (αα) had the lowest total energy. *p*-Bromophenyl-boronate derivative of the synthetic racemate of sarcophytol B, a 13-hydroxy derivative of **1a**, has recently been shown to take αα conformation in crystalline state.[8] It has been reported that the *m*-chloroperbenzoic acid oxidation of sarcophytol A acetate (**1b**) gave both the 7*R*,8*R*/7*S*,8*S*- and 11*R*,12*R*/11*S*,12*S*-epoxide pairs,[1b] together with 3*S*,4*S*-epoxide (3.5%),[1c] in comparable yields (7*R*,8*R*:7*S*,8*S*=35:24, 11*R*,12*R*:11*S*,12*S*=1.3:1.8).[9] Since only the outer face of the



olefin bonds would be attacked in the epoxidation reaction in such systems, this suggested the occurrence of facile conformational interconversion, at room temperature, about the C-7 and C-11 double bonds. The present result is consistent with the product ratio of the epoxidation of **1b**. Compound **1a** was expected to take all four conformers, or at least the three conformers **A**, **B**, and **C**. Comparison of the conformation of crystalline **1a** with these four conformer was not possible due to the unsuitable crystallographic nature of **1a** with low melting point (55-57°C from acetonitrile).



The *p*-bromobenzoate of **1a** failed to crystallize so that the *S*-α-methoxy-α-trifluoromethylphenylacetate (MTPA, **1c**, mp 90-91°C) was subjected to X-ray analysis. The crystal of **1c** belonged to orthorhombic space group, $P2_12_12_1$, with the cellparameter of a = 11.1427 (5), b = 29.0565 (12), c = 8.8222 (8) Å, Z = 4, cell volume = 2856.3Å^3, and D_{calc} = 1.160 gcm^{-3}. Intensity data were measured on an Enraf-Nonius CAD-4 diffractiometer using a graphite-monochromatized Cu*K*α radiation in the ω-scan mode within 2θ less than 140°. A total of 2024 independent reflections were considered as observed, $|F_o|>3\sigma(|F_o|)$. The structure was solved by the direct method with the MULTAN 78 and Monte Carlo method and refined using the full-matrix least-square method to the final *R* factor of 0.0695. The ORTEP drawing of the stereostructure of **1c** confirmed the 14*S* absolute configuration of **1a** as deduced previously by Horeau determination,[10] and by ^{1}H-NMR analysis[11] of the *R*- and *S*-MTPA esters of **1a**.[12] The conformation of **1c** did not correspond to any of the four conformations of **1a** derived by Discover. The C-11 double bond is nearly parallel to the ring plane and H-11 is directed to the center of the ring. Possibly substitution of the hydroxyl proton of **1a** with the bulky MTPA moiety caused significant changes in the overall spatial arrangement of the cembrane ring, and in the crystal packing forces. An NOE was observed between H-11 and H-14 in **1c** which is absent in the ^{1}H-NMR spectrum of **1a**.

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