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Structural and antivirial studies of dipetalactone and its methyl derivative

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HIGHLIGHTS

- Dipyranocoumarins are investigated using theoretical and experimental methods.
- Coumarin fragment is nearly planar and pyran rings adopt a distorted sofa conformation.
- $\pi\cdots\pi$ Interactions stabilize the pairs of centrosymmetric dimers of dipetalactones.
- Biding energy for dimer of dipelactone is about 20 kcal mol^{-1}.
- Compounds resulted weakly cytotoxic against MT-4 cells.

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ABSTRACT

Dipetalactone (6,6,10,10-tetramethyl-6*H*,10*H*-dipyranocoumarin) and its C4-methyl derivative have been synthesized and structurally studied. The structures of these compounds, explored on the basis of 1D and 2D NMR techniques in solution and the DFT/B3LYP calculations for the single molecule of dipetalactone, are in good agreement with those found in the crystals. In the tetracyclic dipetalactones the central coumarin ring systems are nearly planar and the pyran rings adopt a distorted sofa conformation.

Both compounds crystallize with two symmetry independent molecules, **A** and **B**. The π-stacking is the main force stabilizing the pairs of centrosymmetric dimers, **A**·**A** and **B**·**B**. These dimers interact via the intermolecular C—H$\cdots$O and C—H$\cdots\pi$ hydrogen bonds forming three-dimensional supramolecular frameworks which are nearly isostructural. The MP2 calculations reveal that the dimers of dipetalactone only slightly distort during the crystal formation as compared with those in the gas phase. The energy increase upon such a distortion was estimated as ca. 4 kcal mol^{-1}. In addition, the binding energy for the dimer was found to be ca. 20 kcal mol^{-1}.

Dipetalactone and its methyl derivative were examined for cytotoxicity as well as anti-HIV activity.

1. Introduction

Coumarins belong to an important group of naturally occurring compounds used in the folk medicine. The term coumarin is derived from *Coumarouna odorata*, the name of the tonka bean from which coumarin was first isolated by Vogel in 1820 [1]. Its structure was independently deduced by Fittig and Strecker in 1868 [2]. Within this group of derivatives three main coumarin subtypes have been distinguished: simple coumarins, furanocoumarins and pyranocoumarins. The latter are common constituents of the plants of the genus *Calophyllum*, belonging to the Glusiaceae family. The *Calophyllum* genus, a group of tropical trees of ca. 200 species, have been widely used as medicines by natives as a source of various medicines to treat a large group of diseases [3,4]. The data reported in the literature concerning the chemical and biological properties of those plants reveal that they are strongly active against cancer cell lines [3–6] and show anti-human immuno-deficiency virus activity [3,4,7–12]. The authors suggest that these properties are directly related to the presence of dipyranocoumarin moiety. The unique subclass within the HIV-1 non-nucleoside reverse transcriptase inhibitors is formed by the calanolides [3,4,10,11]. Calanolide A was the first natural product identified as active and has recently been investigated in the phase II/III of

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clinical trials [7]. Some other analogues, such as inophyllum B and cordatolide A have also been isolated from the diverse species of the genus *Calophyllum* and identified to be specific HIV-1 reverse transcriptase inhibitors [3,4,7]. Those phytochemicals have the common scaffold – the tetracyclic dipyranocoumarin core, and differ in the substituents at the C4 position (e.g. methyl group in cordatolides, propyl in calanolides, and phenyl in inophyllums). Moreover, each subgroup has characteristic substituents bonded to the **d** ring (cf. Fig. 1; viz. methyl, hydroxyl or carbonyl groups).

Since the natural resources (tropical trees of the Glusiaceae family) rich of such coumarins are limited, these compounds have been widely obtained in the laboratories; e.g. calanolide A has been synthesized and was found to have similar actions to the natural product [13].

The search for the structural data in the Cambridge Structural Database [14] for this group of compounds indicated that to date only one has been described [15]; for the majority of compounds the spectroscopic methods have been used to establish their structure.

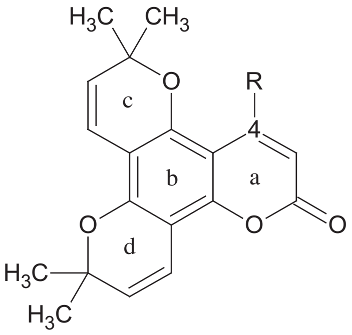
In our laboratory, two simple pyranocoumarins: dipetalactone (6,6,10,10-tetramethyl-6*H*,10*H*-dipyrano[2,3-f;2′,3′-h]coumarin) (**1**) and its C4-methyl derivative (**2**) (Fig. 1) have been obtained. These compounds have been tested in vitro for cytotoxicity and antivirial activity, and structurally characterized by the X-ray crystallography and theoretical calculations (the density functional and MP2 methods). Afterwards, structure elucidation of compounds **1** and **2** in solution was accomplished by the concerted application of 1D (^{1}H, ^{13}C, DEPT) and 2D (HSQC) NMR spectroscopy.

This paper is a continuation of our research on biologically active oxygen heterocyclic aromatic compounds based on coumarin and benzofuran ring [16–18], and the main goal of this study is to describe the stereochemistry and antivirial properties of the investigated compounds being used as conformational scaffold for the synthesis of potent anti-HIV drugs such as e.g. calanolide A.

2. Experimental section

2.1. Synthesis

Chemicals were purchased from Sigma–Aldrich and used without further purification. Dipetalactone (**1**) and 4-methyldipetalactone (**2**) were obtained by the method of Bandaranayake [19], i.e. by the condensation (in the presence of pyridine) of dimethyl acetal of 3-hydroxyisovaleric aldehyde with 5,7-dihydroxycoumarin and 5,7-dihydroxy-4-methylcoumarin [20], respectively. Single crystals of **1** and **2** suitable for X-ray diffraction were prepared by slow evaporation of solvent from an ethanolic solution at room temperature.



1 R = H

2 R = CH_3

Fig. 1. Chemical structure of the investigated compounds **1** and **2**.

2.2. X-ray crystallography

Diffraction data for the crystals of compounds **1** and **2** were collected on a KM4 diffractometer with Cu Kα radiation at room temperature. The structure was solved by direct methods using the SHELXS-97 program and refined by the full-matrix least-squares method on F^2 using the SHELXL-97 program [21]. The non-hydrogen atoms were refined with anisotropic displacement parameters. All H-atoms were positioned geometrically and allowed to ride on the attached atom. The crystallographic data and details of the data collection and refinement are given in Table 1.

CCDC 948790 and 948791 contain the supplementary crystallographic data for compounds **1** and **2**, respectively. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223336033; or e-mail: data_request@ccdc.cam.ac.uk.

2.3. NMR spectroscopy

^{1}H NMR and ^{13}C NMR, as well as the DEPT spectra were recorded at room temperature on a Bruker Avance 300 MHz spectrometer with TMS as an internal standard. The respective data are collected in Table 2.

2.4. Theoretical calculations

The relevant calculations (viz. structures and energies) for molecule **1** (monomer, 41 atoms) and its dimer were initially carried out at the B3LYP level of theory [22] with 6-311++G** basis set [23,24]. DFT/B3LYP in conjunction with VTZ + polarization-quality Pople's basis set is known to be sufficient to accurate calculations of equilibrium geometries of covalently bonded systems. The inclusion of the diffuse basis functions is necessary, though, when binding energies of weakly interacting dimers are to be determined. However, the final energetics is that obtained at the MP2 computational level. Indeed, intermolecular interactions of the dispersive type are poorly accounted at the DFT level with the typical density functionals (like B3LYP). Although dipole-quadrupole ($U^{(\mathrm{int})} \sim r^{-4}$) and quadrupole–quadrupole interactions ($U^{(\mathrm{int})} \sim r^{-5}$) between the substantial parts of molecules seem to predominate, the dispersive interactions ($U^{(\mathrm{int})} \sim r^{-6}$) may not be negligible.

The determination of the full MP2 structures of the molecules as large as those considered in the present work was not practical (the PQS quantum chemistry package [25,26] we used in all calculations, does not include parallel implementation of the MP2 gradient). Thus, the MP2 calculations were carried out using the non-standard procedure described in the Supplementary materials. The final energies were corrected for the basis set superposition error (BSSE) [27]. In addition, the MP2 energies were verified using more extended Dunning's cc-pVTZ basis set [28].

2.5. Cytotoxicity and antiviral assays

CD4 + human T-cells containing an integrated HTLV-1 genome (MT-4) were purchased from American Type Culture Collection (ATCC). Laboratory strain III$_{B}$ of Human Immuno-deficiency Virus type-1 (HIV-1) was obtained from the supernatant of the persistently infected H9/III$_{B}$ cells (NIH 1983).

Cytotoxicity assay was run in parallel with antiviral assays. Exponentially growing MT-4 cells were seeded at an initial density of 1×10^5 cells/mL in 96-well plates in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin G and 100 μg/mL streptomycin. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere, in the absence or presence of serial dilutions of test compounds. Cell viabil-