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An unusual dimer of camptothecin-7-aldehyde

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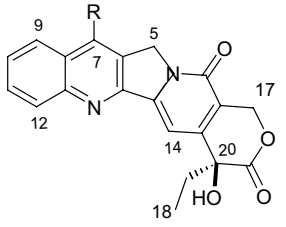
Abstract—An intermolecular aldol reaction of 20*S*-camptothecin-7-aldehyde in the presence of strong bases affords an unusual dimeric compound, the structure and stereochemistry of which was assigned on the basis of NMR analysis and MM2 calculations.

Camptothecin (**1**) is a natural alkaloid endowed with potent cytotoxic activity. In the last two decades much effort has been spent toward the synthesis of new, more active and safer derivatives of camptothecin, that has led to the development of two drugs presently in clinical practice, and of a number of analogues already in various phases of clinical development.[1]

As a part of another work[2] we subjected 20*S*-camptothecin-7-aldehyde (**2**)[3] to Wittig reaction with unstabilized ylides, reaction that requires the use of strong bases. Failure of obtaining the expected alkenes was accompanied by the formation of a new camptothecin derivative (**3**) in variable yield, reaching in some cases 30%. Treatment of **2** with NaH only in THF gave the same product **3**, thus confirming that **3** was formed from **2** by the action of a strong base.[4]



1 R = H
2 R = CHO

Analysis of the NMR spectra (see below) of **3** indicated the disappearance of the aldehyde proton at 11.1 δ and of the corresponding carbonyl carbon at 192.5 ppm, and also of the characteristic singlet of CH_2-5. Conversely, a new OH signal (exchange with D_2O) appeared in the ^1H spectrum at 8.56 δ, slightly coupled with a signal of a CH proton at 6.90 δ (this signal having no other couplings), all the other features of a typical camptothecin spectrum, including the characteristic singlet of the tertiary OH-20 at ca. 6.5 δ, remaining unchanged. The EI mass spectrum showed a molecular peak at 376 m.u., a mass identical with that of the starting aldehyde. A reasonable hypothesis was that the base had extracted a proton from the CH_2-5 group, followed by aldol condensation of the anion onto the aldehyde carbonyl. As an intramolecular condensation had to be ruled out because it would have led to a too much strained fused cyclobutane ring, a possible pathway of formation of **3** was that of an intermolecular condensation, leading to a dimeric compound with an eight-membered ring (Table 1).

The dimeric structure was then confirmed by the FAB mass spectrum that gave a M+1 ion at 753 m.u. The compound gave a tetraacetate **4**, whereas attempts to dehydrate the compound with various agents or to oxidize it to the corresponding diketone gave only untraceable mixtures. NMR spectra of both compounds **3** and **4** were completely symmetric, the signals of the two moieties being superimposable.

A structure such as **3** (apart from stereochemistry) appeared consistent with the spectral data. No example of a similar heavily substituted cyclooctadienediol seems to have been described so far in the literature, nor

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Table 1. NMR data (DMSO-d_6) for compound **3** (camptothecin numbering)

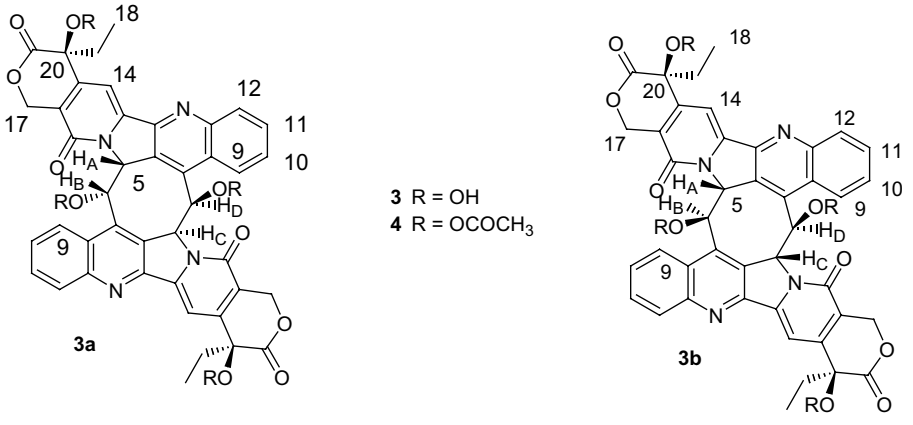
Compound no.	δ_C	δ_H	J (Hz)	HMBC (H→C)
2	150.8	—		
3	143.5	—		
5	70.9	6.63 (s)		CH–OH, C-6, C-7, C-2
6	127.8	—		
7	144.3	—		
8	124	—		
9	129	7.32 (dd)		C-8, C-10
10	131.8	7.54 (ddd)		C-11, C-13
11	122.1	7.28 (ddd)		C-12
12	130	7.69 (dd)		C-8, C-10
13	148.1	—		
14	97.3	6.75 (s)		C-20, C-2, C-3, C-11
15	150.3	—		
16	120.8	—		
16a	157.2	—		
17	65.7	5.53 (d) 5.75 (d)	16.4	C-15, C-16, C-21
18	7.8	0.66 (t)	7.3	C-19, C-20
19	31	1.81 (m)		—
20	72.4	—		
21	172.9	—		
CH–OH	65.7	6.90 (s)		C-5, C-6, C-7, C-8
OH	—	8.56		
OH-20		6.45		

similar reactions have been observed for quinoline or pyridine aldehydes. What remained to be explained was the absence of coupling between the CH-5 and the CH(OH) protons, and the stereochemistry of the compound. In the absence of crystals to submit to X-ray analysis, molecular mechanics calculations (MM2) were performed on the six possible diastereoisomers (not considering the chiral center at C-20) where the hydrogen atoms on the central eight-membered ring are in the relative orientation (in the sequence from H_A to H_D) SSS, AAA, SAA, SSA, SAS, and ASA (where S = *syn*, and A = *anti*, the structure **3a** depicting the SAS isomer, and **3b** the AAA isomer).

From such calculations it appears that only the SAS (**3a**) and the AAA (**3b**) diastereoisomers show minimal energy conformations compatible with a zero coupling constant between H_A and H_B (or H_C and H_D, respectively), the calculated dihedral angles H_A–C–C–H_B and H_C–C–C–H_D being ca. 96° in the former and ca. 88°, respectively, in the latter case. Both these data are consistent with a $J \sim 0$ Hz as obtained from the well-known Karplus equation, whereas for the other diastereoisomers at least one of the angle values is largely different from 90°. Moreover, in the NOESY NMR spectrum, the H-5 proton shows a NOE effect with H-9 (camptothecin numbering). Both structures **3a** and **3b** (or their counterparts where the configurations of the four interested carbons are reversed) are consistent with this result, the distance between the two protons being around 2.0 Å.

Structure **3a** is characterized by a chair eight-membered ring (black in Fig. 1), whereas in **3b** the ring assumes a boat conformation. However, from MM2 calculations **3b** shows a total energy of about 2 kcal less than **3a**, probably also due to the contribution of a strong hydrogen bond between the two OH groups on the central ring, the distance H–OH being of about 1.8 Å, that is possible only for **3b**, the two OH groups being on opposite sites of the ring in **3a** (see, Fig. 1). This is consistent with the unusually low chemical shift of these OH, which appear at 8.56 δ in DMSO in the ^{1}H NMR spectrum. On this basis, we propose the structure **3b** (relative stereochemistry except for 20*S*) for compound **3**.

The facility of generating an anion from a CH_2 group between a pyridine ring and the nitrogen of an amide can be of considerable synthetic interest. The remarkable acidity of such a group was confirmed by H–D exchange NMR experiments, whereby exclusively the protons of this CH_2 group exchanged within 30 min by treatment with excess NaH in THF and quenching with D_2O, not only in camptothecin (**1a**, δ 5.25 in DMSO) but also in the congener alkaloid luotonin A[5] (**5**, δ 5.38 in DMSO).



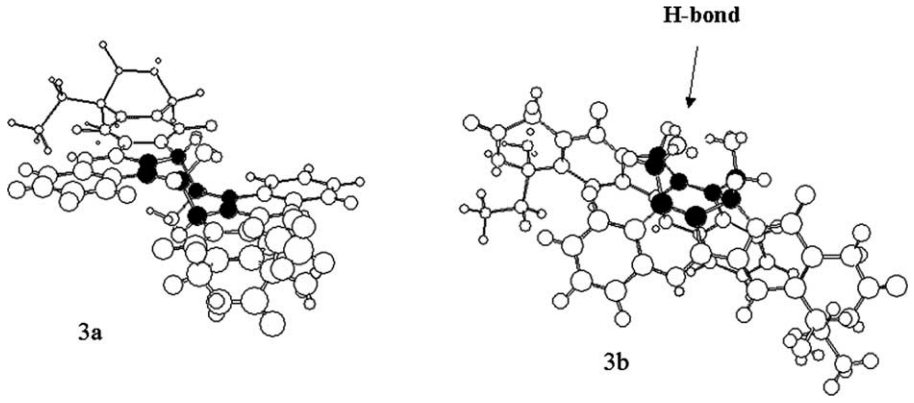
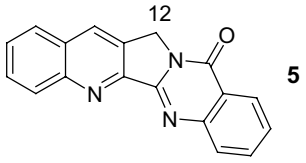


Figure 1. Structures of **3a** and **3b** (from MM2 calculations).



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4. *Experimental*: sodium hydride (9 mg) was added to 8 mL of THF, followed by 120 mg of 20*S*-camptothecin-7-carboxaldehyde.[2] The solution was stirred for 2 h, then evaporated, and the residue subjected to flash chromatography (Silica gel Merck 230–400 mesh) with CH_2Cl_2–MeOH 95:5 as an eluent, to give 37 mg (31%) of **3**, mp 285 °C (dec), $[\alpha]_D$ = +639 (*c* 0.5, $CHCl_3$–MeOH 8:2), EI: *m*/*z* 376(27), 332(56), 317(29), 303(24), 276(43), 275(23), 247(13), 219(23), 218(27), 191(17), 139(100); FAB: *m*/*z* 753 (M+1). Acetylation of **3** (10 mg) with Ac_2O (25 μL) in the presence of TMSOTf (2 μL), followed by usual workup and flash chromatography on silicagel (CH_2Cl_2/MeOH 97:3) gave the tetraacetate **4**; ^{1}H NMR (DMSO-d_6): δ = 0.87 (3H, t, H_3-18), 2.1–2.3 (8H, 1 Ac + H_2-19), 2.40 (3H, s, Ac), 5.57 and 5.90 (2 d, 1H each, J = 16 Hz), 6.76 (2H, s, H_2-5), 6.50 (1H, OH-20), 6.83 (2H, s, H_2-14), 7.40–7.55 (m, 2H arom.), 7.60 (1H, s, C*H*OAc), 7.74 (1H, t, arom.), 7.85 (1H, d, J = 7 Hz, H-12).
When tested in vitro for the cytotoxic activity against a human non-small-cell lung carcinoma cell line, H-460, that overexpresses topoisomerase I, compound **3** appeared only weakly active (IC_{50} = 137 μM) with respect to camptothecin (IC_{50} = 0.39 μM) (data from Dr. F. Zunino, Istituto Nazionale dei Tumori, Milano).
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