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ISOCAMPTOTHECIN

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An expeditious synthesis of the tetracyclic ester, I, *via* the Schiff base of methyl pyruvate has recently been reported.[1] The importance of compound I arises from its conversion to desoxycamptothecin (II) through a lactomethylation reaction with paraformaldehyde.[2] The conjugate base of II is converted to dl-camptothecin (III) by the action of hydrogen peroxide[2]. In this communication, we report the results of a thorough product analysis of the lactomethylation process and describe the synthesis of isocamptothecin (VI) *via* isodesoxycamptothecin (V).

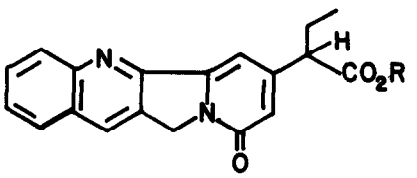
A mixture of I (100 mg. 0.3 mmole) and paraformaldehyde (70 mg, 2.4 mmole) in 10 ml of dioxane containing 60 mg of conc H_2SO_4 and 370 mg of water was heated (sealed tube) for 17 hr at 96°. Workup afforded 45 mg of an acid, IV, which after esterification (MeOH-HCl) and purification returned 37 mg of I. Chromatography of the neutral fraction on silicic acid and elution with chloroform gave, in the following order, 22 mg (35%)[3] of II, 7 mg (11%)[3] of V, mp 274-278°, 11 mg (16%)[3] of homocamptothecin, VII, dec 254-258° and 7 mg (10%)[3] of isohomocamptothecin, VIII, dec 272-275°.

The structure of II follows from the identity of its spectral and chromatographic properties with those of an authentic sample and from its oxidation (1 eq of II, 1.2 eq of potassium-tert-butoxide; 2.5 eq H_2O_2 as a 30% solution, room temperature, 6 hr) to dl-camptothecin (III) in 55% yield[4]. The structure of V is evident from the similarity of its IR, nmr (Figure 1) and mass spectra with those of II. The four significant differences in the nmr spectra ($CDCl_3$) are: (i) the position of the lone pyridone proton (τ3.42 in V), (ii) the chemical shifts of the magnetically non equivalent benzylic methylene protons adjacent to the lactonic oxygen (τ4 45, 4.55 in II, τ3.69, 3.85 in V[5]), (iii) the chemical shifts of the methine protons adjacent to the carbonyl group (τ6.37 in II, τ5.22 in V) and

(iv) the multiplicity of the methylene protons of the ethyl group (magnetically equivalent in II, non-equivalent in V).

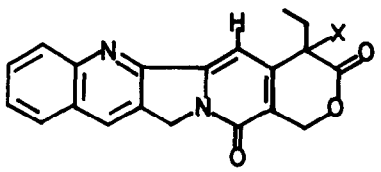
That VII and VIII are derived from the incorporation of an additional formaldehyde unit adjacent to the lactonic carbonyl group, is seen from (i) the presence of OH absorption in their infrared (KBr) spectra, (ii) the absence of the aliphatic methine signals in their nmr spectra and (iii) the appearance of new AB quartets in each of their nmr spectra. For compound VII, where the nmr spectrum is measured in CF_3CO_2D[6], the two doublets are centered at τ5.27 and 5.62 while, in the case of VIII ($CDCl_3$), they appear at τ5.69 and 5.96. The assignment of VII to the normal series and VIII to the iso series is seen most convincingly from (i) the essential identity of their mass spectra with those of II and V[6], respectively and (ii) their transformations to II and V, respectively after pyrolysis at their decomposition temperatures.[6]

Compound V is smoothly converted to isocamptothecin (VI) mp 284-288°, under conditions given above for the transformation of II → III. As is the case with the two desoxy compounds, II and V, camptothecin (III) and isocamptothecin (VI) are clearly different in their chromatographic and spectral properties. The mass spectra of III and VI are shown in Figure 2.



I R = Me

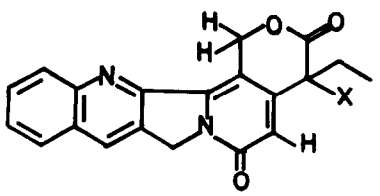
IV R = H



II X = H

III X = OH

VII X = CH_2OH



V X = H

VI X = OH

VIII X = CH_2OH

FIGURE I NMR SPECTRA
M P C 250 MH$_3$

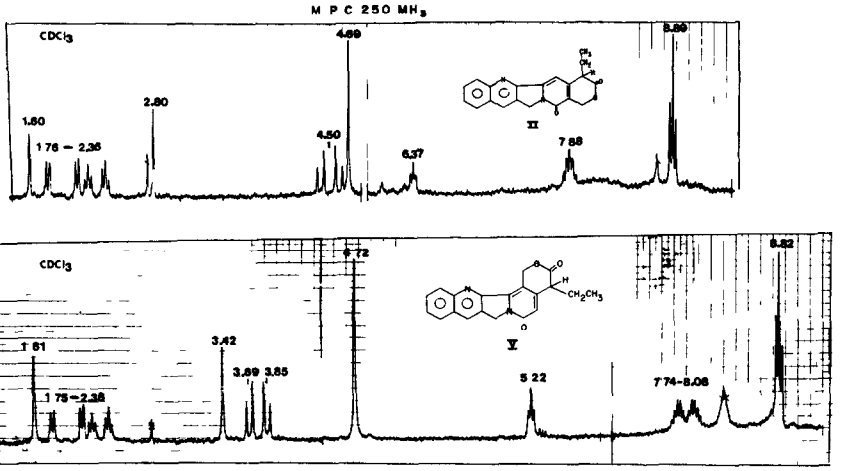
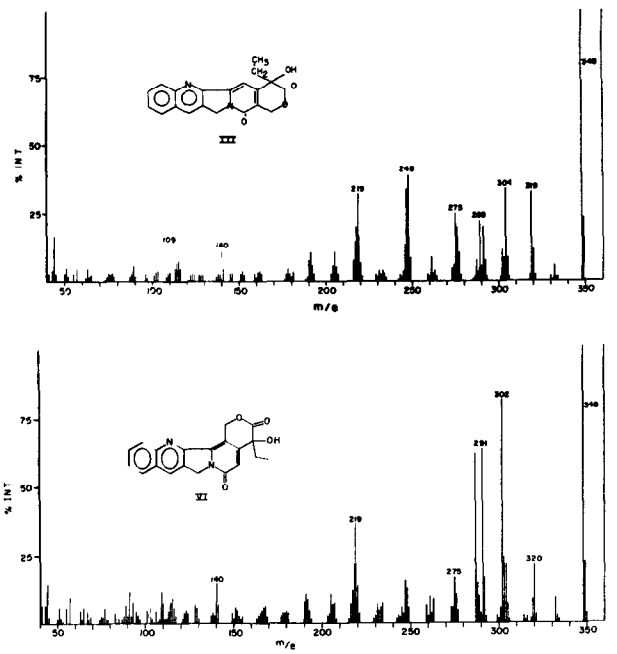


FIGURE II MASS SPECTRA



The effects of the synthetic dl-compounds on the synthesis and degradation of nucleic acids in HeLa cells were studied as previously described [7] Compounds II and III, at concentrations of 2μM, inhibit the synthesis of DNA and RNA by 50%. Similar inhibition in the case of VI required a concentration of 8μM. Both II and III degraded DNA[7] at a concentration of 20μM. At this concentration, the iso-compounds V and VI were significantly less active In the homo-series, the isocompound, VIII was less active than VII in both assays. Compound VII does inhibit DNA synthesis by 50% at a concentration of 2μM. However, it is significantly less active than II and III in the degradation assay. The relationship of the HeLa cell activity to antitumor capabilities, for these compounds, remains to be defined.

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References

1. J. Quick, Ph.D. Thesis, University of Pittsburgh (1972).

2. R. Volkmann, S. Danishefsky, J. Eggler, and D M Solomon, J. Amer Chem. Soc., 93, 5576 (1971).

3. Yields are calculated on the basis of consumed I

4. A sharp improvement relative to previous work[2] was realized by omitting dimethylsulfoxide as a co-solvent.

5. The large downfield shift of these benzylic protons in the iso compound is probably due to deshielding by the quinoline nitrogen (cf. H.P. Husson, C. Thal, P. Potier and E. Wenker J. Org. Chem., 35, 442 (1970).

6. Compound VII and VIII are, to a small extent, converted to II and V, respectively during the interval of measuring their nmr spectra in trifluoroacetic acid This solvent is necessary for solubility reasons in the case of compound VII. The parent ions in their mass spectra (m/e 362) are barely detectable using and A.E.I. - M.S.-9 system but not on an L.B.K.-9,000 spectrometer

7. S. B. Horwitz, C. K. Chang and A. P. Grollman, Mol. Pharmacol , 7, 632 (1971)