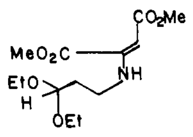

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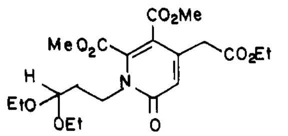

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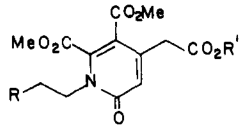
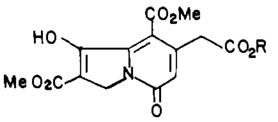


III R = $-\underset{O}{\underset{\|}{C}}-H$; R' = Et

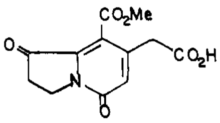
IV R = CO_2H; R' = Et

V R = CO_2Me; R' = Me

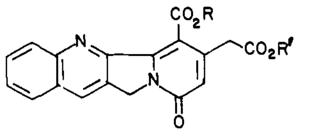


VI R = H

VII R = Me

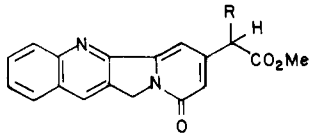


VIII



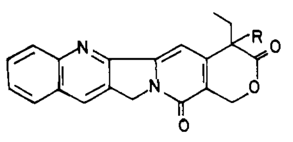
IX R = R' = H

X R = H; R' = Me



XI R = H

XII R = Et



XIII R = H

XIV R = OH

temperature)) gave a 20% yield of ethylated monoester XII,[6] mp 198–200°.[9]

Treatment of XII with paraformaldehyde (in dioxane containing a trace of concentrated H_2SO_4, 100°, 16 hr) gave *dl*-desoxycamptothecin (XIII), mp 258–264° dec, identical with an authentic sample[10] in its chromatographic properties, and nmr, ultraviolet, and mass spectra.[11] With desoxycamptothecin in hand, it soon became clear that this substance undergoes oxidation to camptothecin (XIV) with remarkable facility. For instance, a solution of XIII dissolved in methylene chloride exposed to air soon begins to exhibit, on thin-layer chromatographic analysis, a spot corresponding in its R_f value and mass spectrum to camptothecin. There remained only the translation of this analytical observation to the preparative scale. Our first attempts at this involved passing oxygen through a solution of the anion of XIII (*via* potassium *tert*-butoxide in DMSO–DMF containing 1 equiv of triethyl phosphite). While the formation of camptothecin could be detected, the rate was quite slow and other, as yet unidentified, products were generated.

We then found that treatment of the anion of XIII (*via* potassium *tert*-butoxide in DMSO–BuOH) with 1 equiv of aqueous hydrogen peroxide[12] afforded *dl*-camptothecin, mp 275–277° dec, in 20% yield. The *dl*-camptothecin so produced was identical with the natural product in its mass spectrum and R_f value in a variety of solvents. Its infrared spectrum (Nujol) was superimposable with that of the previously prepared *dl* compound.[4,13]

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[9] The difficulty associated with the ethylation of compound XI led us to rework the synthesis. Treatment of compound II under the same conditions as XI smoothly gave the required ethylated product which was converted to XII using the same series of reactions which converted II into XI.

[10] (a) We thank Dr. Monroe E. Wall of the Research Triangle, Durham, N.C., for furnishing us with a procedure for preparing desoxycamptothecin from the natural product. (b) We thank Dr. Harry B. Wood of the Cancer Chemotherapeutic National Service Center, Bethesda, Md., for furnishing us with a sample of camptothecin.

[11] A minor product of this reaction, mp 258–260°, was also obtained. Its mass spectrum is essentially identical with that of XIII, but it is clearly differentiated from desoxycamptothecin chromatographically. It is tentatively assigned as isodesoxycamptothecin arising from hydroxymethylation at the 5 position of the pyridone followed by lactonization.

[12] *Cf.* G. Büchi, K. E. Matsumoto, and H. Nishimura, *J. Amer. Chem. Soc.*, **93**, 3299 (1971).

[13] We thank Dr. A. Schultz, Columbia University, for comparing our sample with his.

R. Volkmann, S. Danishefsky*
J. Eggler, D. M. Solomon
Department of Chemistry, University of Pittsburgh
Pittsburgh, Pennsylvania 15213

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Studies of the Aromatic Chirality Method. The Optical Rotatory Powers of Di- and Tribenzoate Systems

Sir:

The aromatic chirality method,[1] an extension of the benzoate sector rule,[2] has proven to be a convenient method for determining absolute configurations or conformations. It has successfully been applied to a variety of complex natural products including terpenoids,[3,4] antibiotics,[5,6] sugars,[7] and alkaloids.[1]

According to the molecular exciton theory[8–14] related to the optical rotation properties, the rotational strength R_{ao} of two interacting chromophores (i and j) which exhibit strong $\pi^* \rightarrow \pi$ transitions can be ap-

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[1] N. Harada and K. Nakanishi, *Accounts Chem. Res.*, submitted for publication.

[2] N. Harada, M. Ohashi, and K. Nakanishi, *J. Amer. Chem. Soc.*, **90**, 7349 (1968); N. Harada and K. Nakanishi, *ibid.*, **90**, 7351 (1968).

[3] N. Harada and K. Nakanishi, *ibid.*, **91**, 3989 (1969).

[4] N. Harada and K. Nakanishi, *Chem. Commun.*, 310 (1970).

[5] N. Harada, K. Nakanishi, and S. Tatsuoka, *J. Amer. Chem. Soc.*, **91**, 5896 (1969).

[6] S. Marumo, N. Harada, K. Nakanishi, and T. Nishida, *Chem. Commun.*, 1693 (1970).

[7] N. Harada, H. Sato, and K. Nakanishi, *ibid.*, 1691 (1970).

[8] J. A. Schellman, *Accounts Chem. Res.*, **2**, 266 (1969).

[9] R. Grinter and S. F. Mason, *Trans. Faraday Soc.*, **60**, 274 (1964).

[10] L. S. Forster, A. Moscowitz, J. G. Berger, and K. Mislow, *J. Amer Chem. Soc.*, **84**, 4353 (1962).

[11] B. Bosnich, *Accounts Chem. Res.*, **2**, 266 (1969).

[12] I. Tinoco, Jr., *Advan. Chem. Phys.*, **4**, 113 (1962); I. Tinoco, Jr., and C. A. Bush, *Bio. Symp.*, No. 1, 235 (1964); I. Tinoco, Jr., *Radiat. Res.*, **20**, 133 (1963).

[13] G. Holzworth and P. Doty, *J. Amer. Chem. Soc.*, **87**, 218 (1965).

[14] *Cf.*, A. S. Davydov, "Theory of Molecular Excitons," M. Kasha and M. Oppenheimer, Jr., Transl., McGraw-Hill, New York, N. Y., 1962.

Table I. Uv Data of Para-Substituted Benzoates of Cholesterol and CD Amplitudes of Para-Substituted Dibenzoates of 5α-Cholestane-3β,6β-diol

	Uv					CD	
Substituent	λ_{max}, nm[a]	$\epsilon_{max} \times 10^{-4}$	$r \times 10^8$ cm[b]	σ_{max}, cm^{-1}	$\Delta\sigma$, cm^{-1}	A_{calcd}	A_{obsd}[c]
H	229.5	1.53	0.805	43,572.8	2769.0	−12.7	−15.5
Cl	240.0	2.14	0.992	41,666.6	2665.0	−28.7	−28.5
OMe	257.0	2.04	1.017	38,910.5	2886.1	−23.6	−26.4
NMe_2	311.4	3.16	1.294	32,113.0	2441.0	−58.7	−50.8
CN	240.0	2.46	1.068	41,666.6	2682.1	−38.1	−35.4
NO_2	260.5	1.51	1.034	38,387.7	3869.3	−13.7	−10.7

[a] Uv spectra were measured in ethanol except for the nitrobenzoate (ethanol–dioxane, 24:1). [b] In the methoxy, dimethylamino, and nitrobenzoates, the present transition length value contains that of a $^1A_{1g} \rightarrow {}^1B_{2u}$ transition. However, the effect is considered to be negligible. [c] Solvent, ethanol–dioxane (9:1).

proximated by an electric dipole–electric dipole coupling term as follows:[1] $R_{ao} = \mp(1/2)\pi\sigma_a\vec{R}_{ij}\cdot(\vec{\mu}_{ioa} \times \vec{\mu}_{joa})$, where minus and plus signs correspond to the two singly excited states (β and α) characterized by $E^\beta = E_a + V$, $\Phi_a = (1/\sqrt{2})(\phi_{ia}\phi_{jo} + \phi_{io}\phi_{ja})$, and $E^\alpha = E_a - V$, $\Phi_a = (1/\sqrt{2})(\phi_{ia}\phi_{jo} - \phi_{io}\phi_{ja})$, respectively. The so-called Davydov split is approximated by a dipole–dipole interaction: $V_{ij} = [\vec{e}_i\cdot\vec{e}_j - 3(\vec{e}_i\cdot\vec{R}_{ij})(\vec{e}_j\cdot\vec{R}_{ij})/R_{ij}{}^2]\mu_i\mu_j/R_{ij}{}^3$.

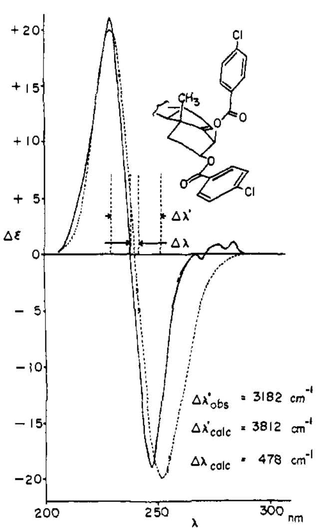

Figure 1. Comparison of calculated (dotted line) and observed (solid line) CD curves for 3β,4β-di-*p*-chlorobenzoyloxycholest-5-ene.

The actual numerical calculation of the CD curve was performed for 3β,4β-di-*p*-chlorobenzoyloxycholest-5-ene, a vicinal glycol dibenzoate. The electric transition moment of the *p*-chlorobenzoate intramolecular charge-transfer band (long axis) was obtained from the integrated intensity of the uv spectrum of cholesterol *p*-chlorobenzoate: $f = 4.32 \times 10^{-9} \int \epsilon(\sigma)d\sigma$; oscillator strength, $f = 0.445$; transition length, $r = 0.9917 \times 10^{-8}$ cm (in ethanol).

The preferred conformation of the dibenzoate moiety was assumed to be as depicted in Figure 1. The Cartesian coordinate of the chromophoric point dipole was considered to be the middle point of the line from chlorine atom to the center of the two oxygen atoms. The interaction energy V was calculated by a point dipole method to give the positive value of +239.1 cm^{-1}, which indicates that the α state is lower than the β state. The computation of the rotational strength for an o → α transition gave $R_{ao}(\alpha) = -3.376 \times 10^{-38}$ cgs unit.

On the other hand, if the CD curve is approximated by a Gaussian curve, the experimental rotational strength $R_{ao}(\alpha)$ is formulated as: $R_{ao}(\alpha) = 2.295 \times 10^{-39}\sqrt{\pi}\Delta\epsilon_{max}\cdot\Delta\sigma/\sigma_{max}$.

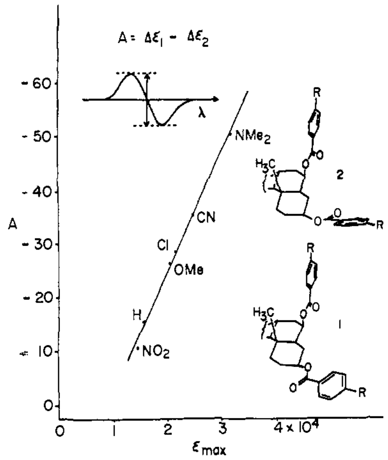

Figure 2. Linear relation between *A* values of para-substituted dibenzoates of 5α-cholestane-3β,6β-diol and ϵ_{max} values of para-substituted benzoates of cholesterol.

The values of $\Delta\sigma$ (1/*e* width) and σ_{max} were obtained from the uv spectrum of cholesterol *p*-chlorobenzoate ($\Delta\sigma$ = 2665 cm^{-1} and σ_{max} = 41666.7 cm^{-1} = 240.0 nm); the calculated value of $\Delta\epsilon_{max}$ is then −129.8. The value for the o → β transition is necessarily $\Delta\epsilon_{max}$ = +129.8, and these two Cotton effects are separated by a Davydov splitting of 478.2 cm^{-1}. Plots of the summation curve of these two CD Cotton effects are shown in Figure 1, together with the observed spectrum. The excellent agreement in position and intensity clearly shows that the aromatic chirality method is applicable to these systems in a facile and straightforward manner.

The following studies on the effect of substituents on split Cotton effects further ascertain the validity of the aromatic chirality method. Various para-substituted dibenzoates of 3β,6β-dihydroxy-5α-cholestane exhibit negative first Cotton effects corresponding to the negative chirality. If a new parameter *A*, characteristic of split type CD Cotton effects, is defined as $A = \Delta\epsilon_1$ (first Cotton effect) − $\Delta\epsilon_2$ (second Cotton effect), the plot of *A* values *vs.* the uv ϵ_{max} values of corresponding para-substituted cholesteryl benzoates is shown to be linear (Figure 2). Thus the *A* values increase with an increase in ϵ_{max} values. Next, the A value for each substituted dibenzoate was actually computed using σ_{max}, $\Delta\sigma$, and r values estimated for the uv spectrum (Table I). In this calculation, two preferred conformations **1** and **2** (Figure 2) were considered, and the coordinates of two point dipoles were assumed to be the same for all substituted dibenzoates. The calculated A values (average value for conformations **1** and **2**)[15] in Table I are in good agreement with observed values and satisfactorily predict the substituent effect. From a practical viewpoint, the chloro- or cyanobenzoates are preferable to other chromophores because of the larger amplitude and ease of preparation.

A similar calculation was performed on a vicinal tribenzoate system, *e.g.*, α-methyl-L-arabinoside 2,3,4-tri-*p*-chlorobenzoate[7] (equatorial–equatorial–axial system). Two apparent Cotton effects are obtained in the tribenzoate system as well, and the longer wavelength Cotton effect sign is in accord with the chiralities of the benzoate chromophores ($A_{calcd} = +122$, $A_{obsd} = +107$).

The symmetrical vicinal tribenzoate (*e.g.*, equatorial–equatorial–equatorial system) does not exhibit a split Cotton effect in agreement with prediction.[7]

As exemplified above for the case of di- and tribenzoates, the aromatic chirality method rests on a sound theoretical background and provides a convenient and versatile method for determining the absolute configurations or conformations of natural products.[1]

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(15) Calculated A values are only slightly affected by conformational rotation: *e.g.*, for *p*-chlorobenzoate, conformations 1 and 2 have $A = -25.8$ and -31.6, respectively.

N. Harada,* S. Suzuki, H. Uda
Chemical Research Institute of Nonaqueous Solutions
Tohoku University, Sendai, Japan, 980
K. Nakanishi*
Department of Chemistry, Columbia University
New York, New York 10027

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Structure of the Antheridium-Inducing Factor of the Fern *Anemia phyllitidis*

Sir:

We assign structure **1** to the antheridium-inducing factor,[1] antheridiogen-An[2] (A_{An}), isolated from culture media of the fern *Anemia phyllitidis* (*Schizaiaceae*).[3] It is the first fern antheridiogen to be characterized, and induces antheridia at 10 μg/l. and also substitutes for light requirement in spore germination at 0.3 μg/l. A total of 1500 flasks, each containing 38 ml of culture medium and cultivated for 53 days, gave 18 mg of pure A_{An}, powder.[4]

The following derivatives of A_{An} [ir (KBr) 1758 (γ-lactone), 1723 cm^{-1} (hindered carboxyl)] were employed in structural studies: methyl ester **2** [(with diazomethane) mass spectrum, M$^+$ peak at 360.1584[5] (calcd for $C_{20}H_{24}O_6$, 360.1573); uv, end absorption; ir (chf), 1761 (γ-lactone), 1729 cm^{-1} (ester)], methyl ester monobenzoate **3** and dibenzoate **4** (with benzoyl chloride–pyridine), and methyl ester monoketone **5** (with chromic anhydride–pyridine). Derivatives **2–4** were submitted to extensive nmr studies (100, 220, and 300 MHz)[6] which established the presence of groups I–III.

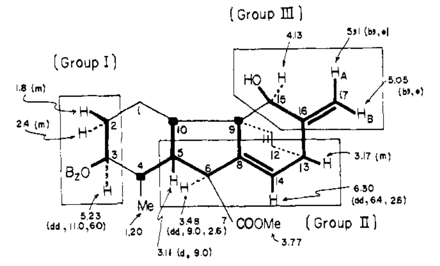

Figure 1. Nmr data of methyl ester monobenzoate **3**: ■, carbons bearing no H; the thick solid and dotted lines connect protons interrelated by nmr.

Groups I, II, and III (Figure 1). Carbinyl proton 3-H[7] (3.74 ppm in ester **2**) is axial ($J_{3,2ax}$ 11 Hz) and is coupled to two other adjacent protons. The system 5-H(ax)–6-H(ax)–14-H(olefinic)–13-H was deduced in a straightforward manner; the 13-H is further coupled to proton(s) around 1.5 ppm. Carbinyl proton 15-H (5.87 ppm in dibenzoate **4**) is weakly coupled to the two exocyclic protons (decoupling). The presence of group III is ascertained from the data of monoketone **5**: uv (EtOH) 217 nm (ϵ 4750); nmr (CDCl$_3$, Fourier transform) H_A at 5.78 (br, s) and H_B at 5.21 ppm (br, s).

Derivation of Planar Structure. Irradiation at 3.17 ppm (13-H) in monobenzoate **3** caused a small but definite increase in the 17-H_B signal area (*ca.* +2.7%) (at 100 MHz, also at 300 MHz). Observation of this crucial NOE allows one to link group II and group III so that they comprise one continuous proton system as in Figure 1. A methyl singlet was present at 1.17–1.28 ppm in all derivatives. Evidence for attachment of this methyl at C-4 and presence of a 6–5–6 ring system was established by microselenium dehydrogenation of A_{An} to yield 1-methylfluorene (purified by sublimation), which was compared with an authentic sample by uv, tlc with several solvents, and Fourier transform nmr. Evidently the allylic alcohol moiety is lost upon selenium dehydrogenation.

The diaxial relation of 5-H and 6-H ($J_{5,6}$ = 9.0–9.6 Hz) in all derivatives **2–5** precludes involvement of C-6 in any bridged system, and hence the methoxycarbonyl group must be attached to C-6. Because C-10 bears no hydrogen, the γ-lactone then can only be connected between C-4 and C-10, with its carbonyl terminal at C-4, as deduced from the 4-Me chemical shift (1.17–1.28 ppm in **2–5**; 1.13–1.22 ppm in models **6–9**).

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(1) W. Döpp, *Ber. Deut. Bot. Ges.*, **63**, 139 (1950).

(2) U. Näf, *Nature* (*London*), **184**, 798 (1959).

(3) M. Endo, K. Nakanishi, and U. Näf, *Science*, submitted for publication.

(4) A_{An} and derivatives were all purified by tlc and the purity was checked by nmr and mass spectral data; due to scarcity of material, no attempts were made to crystallize the compounds.

(5) We thank Dr. G. van Lear, Lederle Laboratories, for the full high-resolution mass spectrum of methyl ester 2 (measured with a MS-9 instrument).

(6) J values were verified by decoupling experiments. We thank Mr. I. Miura for the measurements carried out at Columbia University, and Mr. L. Cary for measurments taken at 300 MHz at Varian Associates.

(7) In view of the close relation between gibberellins and A_{An}, the gibberellane (gibbane) nomenclature is adopted. The numbering system is based on the third revision of diterpene nomenclature as proposed to IUPAC by J. W. Rowe, USDA, Madison, Wisc.