sults from (3*S*)-phosphoglycerate-3-*t* gives rise to (6*R*) shikimate-6-*t*. Therefore, the DAHP formed in the reaction is labeled in the *pro*-3*S* hydrogen,[14] *i.e.*, the attack at C-3 of phosphoenolpyruvate is at the side of the plane viewed at which the substituents phosphate, carboxyl, methylene appear in counterclockwise order (*si* face[15]). This information is, of course, of no mechanistic significance, but it may be of interest from the viewpoint of enzyme evolution. Rose and his coworkers[16] recently studied different phosphopyruvate carboxylases (EC 4.1.1.31, EC 4.1.1.32, EC 4.1.1.38) and found that in all three cases the reaction involves ***si*** attack at C-3 of phosphoenolpyruvate.

Acknowledgments. We are indebted to Dr. B. D. Davis, Harvard Medical School, for providing us with a culture of *E. coli* mutant 83-24 and in particular to Dr. Howard Zalkin, Department of Biochemistry, Purdue University, for gifts of bacterial cultures as well as enriched preparations of DAHP synthetase and anthranilate synthetase. Thanks are due to Dr. I. A. Rose, Institute for Cancer Research, Philadelphia, Pa., for communicating to us his unpublished results. Partial support of this work by U. S. Public Health Service Grant No. FR 05586 is gratefully acknowledged.

(14) Note that the 3*S* hydrogen of DAHP is sterically equivalent to the 6*R* hydrogen of shikimate. The possibility that an inversion takes place at this carbon atom during the conversion of DAHP into shikimate, although not strictly excluded, is considered to be extremely remote.

(15) K. R. Hanson, *J. Amer. Chem. Soc.*, 88, 2731 (1966).

(16) I. A. Rose, E. L. O'Connell, P. Noce, M. F. Utter, H. G. Wood, J. M. Willard, and T. G. Cooper, unpublished results.

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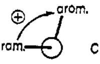
A Method for Determining the Chirality of Two Aromatic Chromophores and the Absolute Configurations of Chromomycin A_3 and Related Antibiotics

Sir:

We have recently developed a method for determining the chiralities between hydroxyl groups[1,2] which is based on the splitting of the original Cotton effect associated with the benzoate chromophore. Thus, the isolated benzoate group shows a Cotton effect at 225 nm ($\Delta\epsilon \sim 3.5$),[3,4] whereas, in the case of interacting benzoate groups, this is subject to a Davydov splitting[5,6] and is characterized by two Cotton effects centered at 233 and 219 nm ($\Delta\epsilon \sim 10$–15); the sign of the longer wavelength Cotton effect (first Cotton) is in accord with the screwness of the benzoate groups, which is defined as positive when represented as in **1** (right-handed screw).

This "dibenzoate chirality rule" can now be extended to encompass other aromatic chromophores for which directions of the two interacting transitions are known; it therefore provides an extremely powerful method for determining the absolute configurations or conformations of natural products which already possess or are readily convertible into derivatives possessing suitable chromophores.



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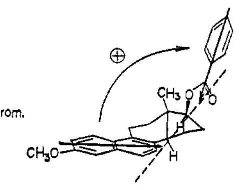

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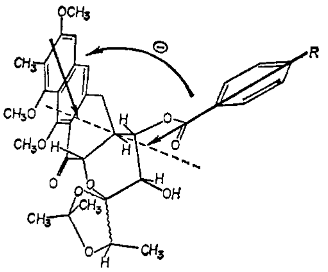

11, R; H
12, R; OMe

The nonempirically calculated absolute signs of Cotton effects resulting from interacting benzoate chromophores agree with the chirality of the two aromatic groups.[1,7] It was thus expected that the treatment employed in the case of interacting benzoate groups could be extended to other groups as well. This is confirmed by the CD datum of 17β-dihydroequilenin 3-methyl ether 17-benzoate (**3**), mp 167–169°[8] (Figure 1). The free 17β-ol **2** exhibits Cotton effects centered at 330, 280, and 226 nm corresponding to the uv maxima of the methoxynaphthalene group. The $^1A \rightarrow {}^1B_b$ transition (long axis) at 230 nm[9] (Figure 2) is strongly coupled with the benzoate transition at 230 nm, and consequently the CD spectrum has two strong Cotton effects at 235 and 220 nm (Figure 1). Significantly, the positive sign of the first Cotton effect is in agreement with the chirality between the long axis of the naphthalene moiety and the benzoate group (or to a first approximation, direction of the C_{17}–O bond; see **4**). The two other transitions, $^1A \rightarrow {}^1L_a$ (short) at 280 nm and $^1A \rightarrow {}^1L_b$ (long) at 330 nm, were not affected by the dipole–dipole coupling with the benzoate groups (Figure 1) because (a) the bands are located far from the 230-nm benzoate transition, and (b) their electric transition moments are relatively small, and therefore the rotational strength which is proportional to the electric transition moment is also small.[1]

The present method was next applied to chromomycin A_3 (**5**) belonging to the chromomycin[10] and olivomycin[11,12] group of antitumor antibiotics, the absolute configuration of which remains to be established.[13]

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(3) N. Harada, Mo. Ohashi, and K. Nakanishi, *J. Amer. Chem. Soc.*, **90**, 7349 (1968).

(4) N. Harada and K. Nakanishi, *ibid.*, **90**, 7351 (1968).

(5) A. S. Davydov, "Theory of Molecular Excitons," translated by M. Kasha and M. Oppenheimer, Jr., McGraw-Hill Book Co., Inc., New York, N. Y., 1962.

(6) J. A. Schellman, *Accounts Chem. Res.*, **1**, 144 (1968).

(7) See also S. F. Mason in "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," G. Snatzke Ed., Heyden, London, 1967, Chapter 4.

(8) Prepared by benzoylation of the 3-methyl ether: W. E. Bachmann and A. S. Dreiding, *J. Amer. Chem. Soc.*, **72**, 1323 (1950). The 17β configuration has been confirmed by X-ray analysis for estradiol 3-*p*-bromobenzoate: Y. Tsukuda, T. Sato, M. Shiro, and H. Koyama, *J. Chem. Soc., B*, 1387 (1968).

(9) H. H. Jaffé and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," John Wiley & Sons, Inc., New York, N. Y., 1962.

(10) M. Miyamoto, Y. Kawamatsu, K. Kawashima, M. Shinohara, K. Tanaka, S. Tatsuoka, and K. Nakanishi, *Tetrahedron*, **23**, 421 (1967), and previous papers.

(11) G. P. Bakhaeva, Yu. A. Berlin, O. A. Chuprunova, M. N. Kolosov, G. Yu. Peck, L. A. Piotrovich, M. M. Shemyakin, and I. V. Vasina, *Chem. Commun.*, 10 (1967), and previous papers.

(12) The olivomycins differ from chromomycin in that they lack the 7-Me group in the aglycone. The various chromomycins and olivomycins differ in the number and type of sugar residues attached to C-2 and C-6.

(13) Previous studies are not well grounded and have led to conflicting results. The absolute configuration of the chromomycins[14] rested

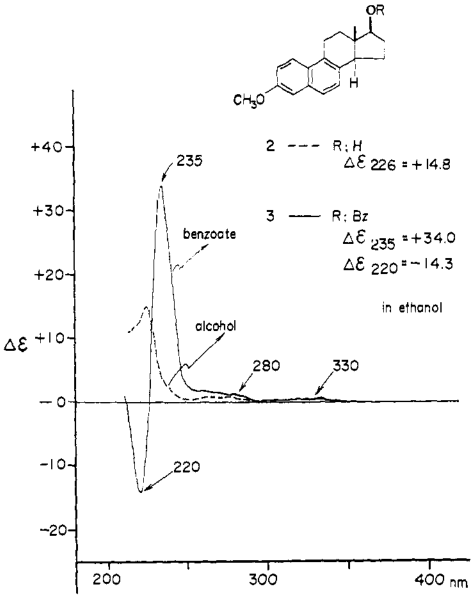
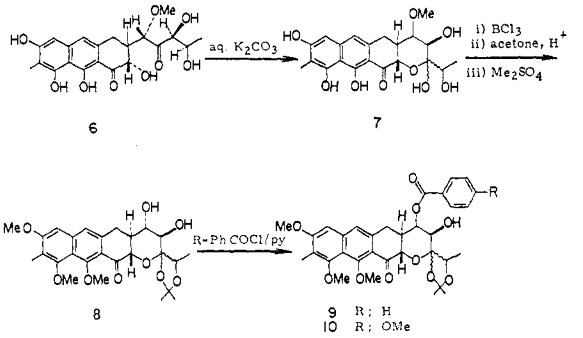


Figure 1. CD spectra of 17β-dihydroequilenin 3-methyl ether **2** and its benzoate **3**.

The aglycone, chromomycinone (**6**), was isomerized to isochromomycinone (**7**)[16] of established relative configuration,[14] and this was finally converted to the benzoate **9**, mp 135–137° dec, molecular ion at *m/e* 592.2353 (calcd 592.2303), and *p*-methoxybenzoate **10**, mp 142–144° dec,[17] molecular ion at *m/e* 622.2478 (calcd 622.2408).



The uv peaks due to the naphthalenoid moiety in glycol **8** can be readily assigned to the various transitions by comparing the spectrum with related compounds (Figure 2). The ORD of glycol **8** consists of weak negative and weak positive Cotton effects (apparent $a = -334$). On the other hand, the ORD of benzoate **9** is a summation curve of two Cotton effects of opposite signs separated by Davydov splitting,[18] which are centered around 270 (apparent $a = -1140$) and 230 nm (Figure 3). This clearly shows that the Cotton effects in the ORD of benzoate **9** originate from the dipole–dipole interaction between the electric transition moments of the benzoate chromophore (230 nm, $\epsilon \sim 14{,}000$) and naphthalenoid chromophore in the glycol **8** (270 nm, ϵ 57,200). As the sign of the first Cotton effect is negative, the long axis transitions of the two chromophores interact as depicted in **11** (negative chirality), and, consequently, the total structure of chromomycin A_3 should be represented by **5**.[19]

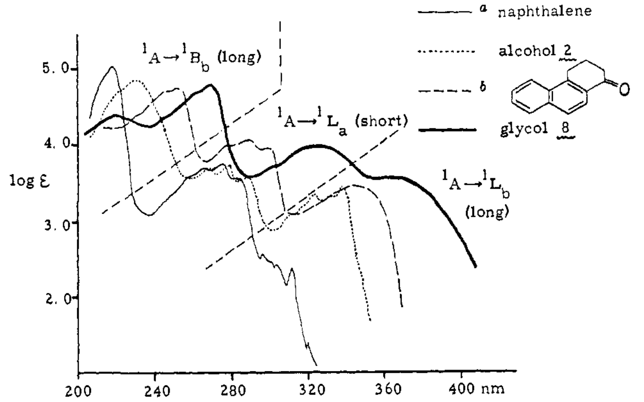


Figure 2. Uv spectra of pertinent naphthalenoid compounds in ethanol. (a) Adapted from W. V. Mayneord and E. M. F. Roe, *Proc. Roy. Soc.* (London), **A152**, 299 (1935). (b) Adapted from R. Hursgen and U. Rietz, *Tetrahedron*, **2**, 271 (1958).

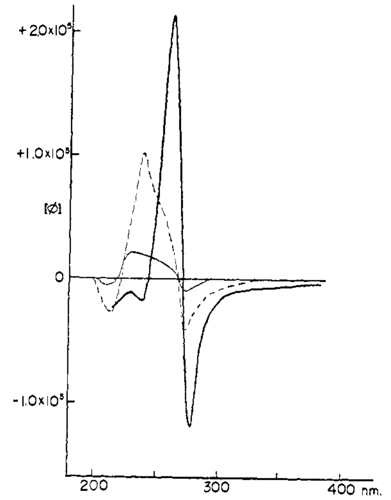
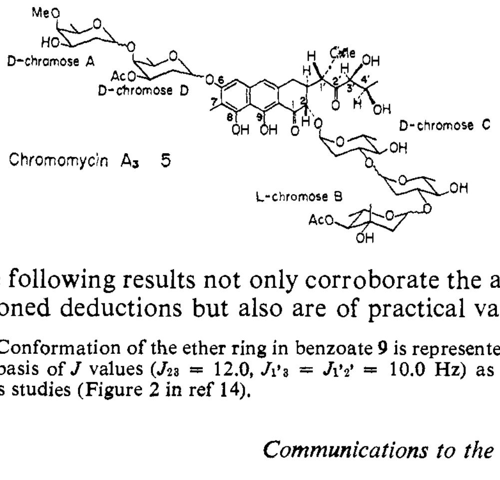


Figure 3. ORD spectra of chromomycin derivatives in ethanol: (—) glycol **8**, (- - - -) benzoate **9**, (—) *p*-methoxybenzoate **10**.



The following results not only corroborate the above-mentioned deductions but also are of practical value as

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on application of Brewster's benzoate rule[15] to 2,6,8,9-tetrabenzoates of the aglycone derivative, thus neglecting the interaction between benzoate groups, whereas that of the olivomycins[11] was based on rotatory dispersion comparisons (wavelength unspecified) between an anthracene derived from the aglycone and a mandelic ester, thus comparing two unrelated chromophores. The present conclusion is in agreement with that of the Russian workers.

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(15) J. H. Brewster, *ibid.*, **13**, 106 (1961).

(16) M. Miyamoto, K. Morita, Y. Kawamatsu, S. Noguchi, R. Marumoto, M. Sasai, A. Nohara, Y. Nakadaira, Y. Y. Lin, and K. Nakanishi, *ibid.*, **22**, 2761 (1966).

(17) Spectroscopic data of compounds **7** to **10** were in agreement with the structures shown.

(18) The ORD of two Cotton effects separated from each other by a Davydov splitting are characterized by a curve consisting of peak–trough–peak or trough–peak–trough; see Figure 1 in ref 1 and Figure 5 in ref 6.

(19) Conformation of the ether ring in benzoate **9** is represented by **11** on the basis of *J* values (J_{23} = 12.0, $J_{1'3}$ = $J_{1'2'}$ = 10.0 Hz) as well as previous studies (Figure 2 in ref 14).

they indicate that interaction between two groups can be greatly enhanced by appropriately modifying the chromophores. The *p*-methoxybenzoate chromophore in **10** would be expected to interact much more efficiently with the naphthalenoid chromophore because its band at 256 nm ($\epsilon \sim 18{,}000$) is located closer to the corresponding naphthalenoid band, and according to the molecular exciton model the chromophoric interaction would be favored by similar excitation energies. As shown in Figure 3, this expectation is verified, and the apparent amplitudes of the ORD Cotton effects are enhanced about three times ($a = -3326$); the CD curve also consists of two strongly interacting Cotton effects at 272 ($\Delta\epsilon$ −61.1) and 250 nm ($\Delta\epsilon$ +39.9).

Since the Cotton effects associated with dipole–dipole interaction between two aromatic chromophores are extremely strong[1,7] and only the directions of transition moments need be considered, the present method should be widely applicable to the determination of the chiralities between benzoate groups and/or other aromatic chromophores.[20]

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Quenching of Acylnitrile Singlets by Piperylene

Sir:

Singlet quenching of aromatic compounds by conjugated dienes is well documented and apparently proceeds by an electronic–vibrational energy-transfer mechanism.[1] A purely electronic mechanism is ruled out on energetic grounds. The corresponding quenching of the singlet states of carbonyl compounds has not been observed,[2–4] although the rates of intersystem crossing (the major mode of deactivation of the singlet state) are not prohibitive for diffusional quenching of alkyl ketone[5] and diketone[6] singlet states. In the present communication we note and stress that *singlet quenching by dienes is not restricted to aromatic systems.*

In the course of an investigation of the photoreactions of acylnitriles, we have found that in the several solvents employed the "apparent" intersystem crossing yield,[7] $(\varphi_{ic})_a$, for pyruvonitrile (CH_3COCN) varies with the concentration of *cis*-1,3-pentadiene, better known as *cis*-piperylene (Figure 1 and Table I).[8] Analytical monitoring involves only the *cis* → *trans* isomerization of piperylene. The results clearly show that the olefin is capturing the precursor to the state responsible for photosensitization leading to acceptor isomerization (*i.e.*, the lowest triplet state). Furthermore, this precursor does not itself cause chemical change of piperylene.[9] Formation of oxetanes and/or piperylene cyclodimers is of minor, if any, significance since such products would have been observed.[9] All of the above observations indicate that the precursor being captured is the singlet state of pyruvonitrile and are in agreement with the observations on singlet quenching of aromatic compounds.[1d] A simple, purely electronic singlet–singlet energy transfer mechanism for the system under consideration is ruled out on the same basis as it is by Hammond and Stephenson[1d] for the aromatic systems. The absorption maximum for the first singlet of pyruvonitrile is at 310 nm in cyclohexane, the 0–0 band being 40–50 nm to the red of this. For *cis*-piperylene λ_{max} (cyclohexane) is near 230 nm, the 0–0 band lying about 20 nm to the red, at about 250 nm. Pyruvonitrile emits relatively strong phosphorescence (at 77°K in an EPA glass), indicating a triplet energy of 72 ± 2 kcal/mol. No fluorescence was detectable either at 77°K or at ambient temperatures. In many respects acylnitriles are spectroscopically very much like aliphatic ketones and α-keto acids and esters—a fact of some consequence

Table I. Determinations of the "Apparent" Intersystem Crossing Yields $(\varphi_{ic})_a$

Solvent	Piperylene concn, *M*	$(\varphi_{ic})_a$	$(\varphi_{ic})_a^{-1}$
Acetic acid	0.0186	0.376	2.66
	0.0419	0.33	3.03
	0.0910	0.40	2.50
	0.0964	0.42	2.38
	0.258	0.255	3.92
	0.473	0.195	5.14
	0.950	0.12	8.3
	1.55	0.09	11.1
Acetonitrile	0.138	0.162	6.16
	0.190	0.0765	13.1
	0.278	0.0565	17.7
	0.478	0.039	25.6
Carbon tetrachloride	0.0967	0.157	6.36
	0.345	0.126	7.94
	0.619	0.0855	11.7
Isopentane	0.209	0.086–0.103	11.6–9.7
	0.198	0.0382	26.2
	0.408	0.0298	33.6

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[1] (a) L. M. Stephenson, D. G. Whitten, G. F. Vesley, and G. S. Hammond, *J. Am. Chem. Soc.*, 88, 3665 (1966); (b) S. L. Murov, R. S. Cole, and G. S. Hammond, *ibid.*, **90**, 2957 (1968); (c) S. Murov and G. S. Hammond, *J. Phys. Chem.*, **72**, 3797 (1968); (d) L. M. Stephenson and G. S. Hammond, *Pure Appl. Chem.*, **16**, 125 (1968); (e) R. S. Cooke and G. S. Hammond, *J. Am. Chem. Soc.*, **90**, 2958 (1968).

[2] P. J. Wagner and G. S. Hammond, *ibid.*, 87, 4009 (1965); P. J. Wagner and G. S. Hammond, *ibid.*, 88, 1245 (1966).

[3] T. J. Dougherty, *ibid.*, 87, 4011 (1965).

[4] R. E. Rebbert and P. Ausloss, *ibid.*, 87, 5569 (1965).

[5] For calculations concerning the rate of intersystem crossing of acetone from its fluorescence spectrum, see R. F. Borkmann and D. R. Kearns, *J. Chem. Phys.*, **44**, 945 (1966). However, recent results indicate that there may be complications with regard to the normally observed fluorescence emission of alkyl ketones: M. O. Sullivan and A. C. Testa, *J. Am. Chem. Soc.*, **90**, 6245 (1968).

[6] N. J. Turro and R. Engel, *ibid.*, **90**, 2989 (1968).

[7] As measured by the method of A. A. Lamola and G. S. Hammond, *J. Chem. Phys.*, **43**, 2129 (1965).

[8] All photoreactions were performed at ambient temperatures (28°) using a Hanovia medium-pressure mercury lamp surrounded by a 3100-Å filter (K_2CrO_4, 0.2 g/l.; K_2CO_3, 10 g/l.). The samples were degassed, sealed in Pyrex tubes, and held in a Southern New England Ultraviolet MGR-500 merry-go-round apparatus. Pyruvonitrile concentration was invariably 0.10 *M*.

[9] The photostationary state (pss = $[c]/[t]$) is independent of olefin concentration; *no measurable loss of piperylene or pyruvonitrile has been found.*