The Structure of Costatolide[1a,b]

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Costatolide, a new naturally occurring coumarin, has been shown by chemical and spectroscopic means to have the structure 17. The structure derived from the deductive evidence was confirmed by the synthesis of its dihydro derivative. Detailed analysis of the n.m.r. spectra of costatolide and its derivatives allowed the assignment of the relative configurations of the three asymmetric centers.

As part of our continuing study of the products of the plant family Guttiferae,[2] we have investigated the resin of *Calophyllum costatum* Bail. In 1960 one of us (G. H. S.) collected 18 g. of crude exuded resin, somewhat mixed with bark, from a specimen growing in northern Queensland, Australia. Crystallization of the crude resin from benzene showed it to consist of at least 50% of a new compound, m.p. 181–182°, which we have named costatolide.

Structure.—Costatolide is colorless, neutral, and optically active; it was assigned the formula $C_{22}H_{26}O_5$ on the basis of combustion analyses and a molecular weight of 370, found mass spectrometrically. It is photosensitive: solutions, especially in chloroform, rapidly turn red when exposed to sunlight.

The derivatives of costatolide important to the following discussion are those obtained by reduction and oxidation. Hydrogenation of costatolide in methanol using Adams catalyst led to absorption of 1 mole of hydrogen and the formation of dihydrocostatolide, $C_{22}H_{28}O_5$, m.p. 159–160°. If acetic acid was used as the solvent, 2 moles of hydrogen was consumed and the resulting product, deoxydihydrocostatolide, m.p. 115–120°, proved to have the formula, $C_{22}H_{28}O_4$, corresponding to the loss of an oxygen atom from dihydrocostatolide. Finally, oxidation of dihydrocostatolide with Jones reagent[3] led to the formation of the ketone, oxodihydrocostatolide, $C_{22}H_{26}O_5$, m.p. 163–165°.

The infrared spectrum of costatolide shows bands corresponding to hydroxyl (2.75 μ), carbonyl (5.74 μ), and aromatic (6.18 and 6.28 μ) groups. The ultraviolet spectrum was indicative of the presence of one or more aromatic chromophores, but could not be assigned out of hand to a particular class. The spectra of dihydrocostatolide and deoxydihydrocostatolide, however, are extremely similar to those reported[4] for a number of 5,7-dioxygenated coumarins (1) and suggested that such a chromophore might be present in

1   2

these derivatives. The proposal of this system appeared quite reasonable in view of the isolation of a number of coumarins, all containing oxygen atoms at positions 5 and 7, from other species of *Calophyllum*[5,6] and related genera.[7,8] Confirmation was found in the observation that the spectrum of oxodihydrocostatolide, although markedly different from that of dihydrocostatolide, is in turn very similar to those of coumarins containing a 6- or 8-acyl group in addition to the 5- and 7-oxygen substituents.[4,7]

On the other hand, costatolide is not a phenol, since it shows no spectral shift when studied in alcoholic base and gives no ferric chloride test. Furthermore, no methoxyl peaks could be found in the n.m.r. spectrum (Fig. 1) in the region of τ 6. Thus the oxygen atoms whose presence was suggested above must occur either as complex ethers or as portions of additional heterocyclic rings. The total absence of any peaks attributable to aromatic protons in the n.m.r. spectrum of costatolide and its derivatives and the resulting necessity of having two further substituents on the aromatic portion of 1 led us to favor the latter. The empirical formula of dihydrocostatolide is also consistent with the addition of two saturated rings to 1.

That one of the extra rings in costatolide is part of a 2,2-dimethylchromene system (2) was suggested by several lines of evidence. The marked change which occurs in the ultraviolet spectrum of costatolide on hydrogenation showed that the reducible double bond is conjugated with the aromatic nucleus. The n.m.r. spectrum of costatolide shows a well-defined pair of doublets at τ 3.24 and 4.40 ($J = 9.6$ c.p.s.) with intensities corresponding to one proton each. The identification of these peaks with the protons of the double bond was confirmed by their replacement in the spectrum of dihydrocostatolide (Fig. 2) by triplets at τ 7.35 and 8.22 ($J = 6$ c.p.s.). The fact that one of the methylene groups resulting from the saturation of the double bond shows proton signals at τ 7.35, in the region associated with benzylic protons, is in accord with our view that the double bond is conjugated with the ring. The n.m.r. spectra of a number of 2,2-dimethylchromene derivatives have been reported,[9–12] and all show
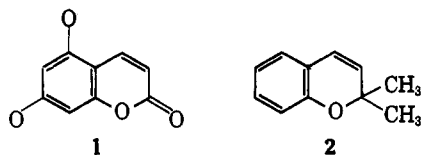
(1) (a) Presented in part at the 18th Northwest Regional Meeting of the American Chemical Society, Bellingham, Wash., June, 1963. (b) Supported in part by Grant CA-04082 from the National Institutes of Health and by a grant from the Institute of Forest Products, University of Washington. (c) Abstracted in part from the Ph.D. Thesis of K. L. Stevens. (d) National Institutes of Health Predoctoral Fellow, 1964.

(2) For previous papers, *cf.* R. A. Alden, G. H. Stout, J. Kraut, and D. F. High, *Acta Cryst.*, **17**, 109 (1964); G. H. Stout, V. F. Stout, and M. J. Welsh, *Tetrahedron*, **19**, 667 (1963).

(3) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(4) R. A. Finnegan, B. Gilbert, E. J. Eisenbraun, and C. Djerassi, *J. Org. Chem.*, **25**, 2169 (1960).

(5) A. Ormancey-Potier, A. Bugas, and E. Lederer, *Bull. soc. chim. France*, 577 (1951).

(6) J. Polonsky and R. Toubiana, *Compt. rend.*, **242**, 2877 (1956).

(7) C. Djerassi, E. J. Eisenbraun, B. Gilbert, A. J. Lemin, S. P. Marfey, and M. P. Morris, *J. Am. Chem. Soc.*, **80**, 3686 (1958).

(8) D. P. Chakaborty and P. K. Bose, *Proc. Natl. Inst. Sci. India, Pt. A*, **26**, 1 (1960).

(9) B. F. Burrows, W. D. Ollis, and L. M. Jackman, *Proc. Chem. Soc.*, 177 (1960).

(10) M. L. Wolfrom, F. Komitsky, G. Fraenkel, J. H. Hooker, E. E. Dickey, P. McWain, A. Thompson, P. M. Mundell, and D. M. Windrath, *Tetrahedron Letters*, 749 (1963).

(11) C. S. Barnes, J. L. Occolowitz, N. L. Dutta, P. Madhavan Nair, P. S. Phadke, and K. Venkataraman, *ibid.*, 281 (1963).

two doublets ($J \sim 10$ c.p.s.), one at τ 3.2 to 3.5 and the other at τ 4.3 to 4.6. Furthermore, the six methyl protons appear at a singlet between τ 8.51 and 8.62. Costatolide shows a six-proton singlet at τ 8.52, which shifts as expected to higher field (τ 8.61) in dihydrocostatolide.

Chemical confirmation for this structural suggestion was found in the identification of both acetone and acetaldehyde as their dinitrophenylhydrazone derivatives following the degradation of costatolide with dilute base. The isolation of acetone under such conditions has been regarded as proof for the presence of a dimethylchromene system,[13] and acetaldehyde has also been obtained in cases in which it was conserved by being removed continuously from the reaction system.[12,14,15]

The mass spectrum of costatolide is also consistent with structure 2 since it shows a peak at $m/e = 355$ (M-15) which is four times as intense as that of the parent ion. These results are in agreement with the observed[11] ready formation of stable benzopyrylium ions from 2,2-dimethylchromenes.

The only other signal which appears below τ 5 in the n.m.r. spectra of costatolide and its derivatives is a singlet at τ 3.96 to 4.1. The protons on the double bond of the coumarin system have been found to fall in the ranges ca. τ 3.5 to 4 (C-3) and τ 1.9 to 2.4 (C-4) with $J \sim 10$ c.p.s.[16]; thus the appearance of only a high-field singlet indicates that in costatolide C-4 is substituted.

The base degradation of coumarins bearing a group R at C-4, with the formation of methyl ketones, RCO-Me, is well known.[17] Thus, when deoxydihydrocostatolide was subjected to strong base hydrolysis, 2-pentanone, identified by v.p.c. and by paper chromatography of its dinitrophenylhydrazone, was obtained. On the strength of this result, the C-4 substituent was identified as an n-propyl group.

The n.m.r. spectrum of costatolide is also consistent with the presence of an n-propyl chain attached to C-4. A triplet at τ 7.06 (2H) may be identified with the

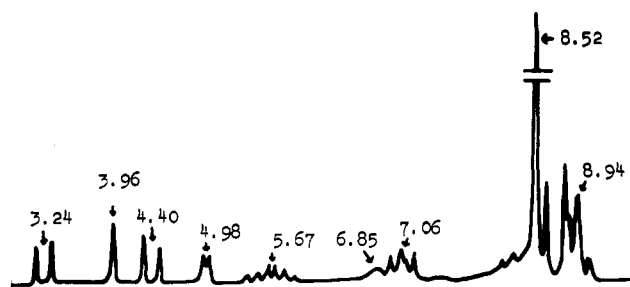

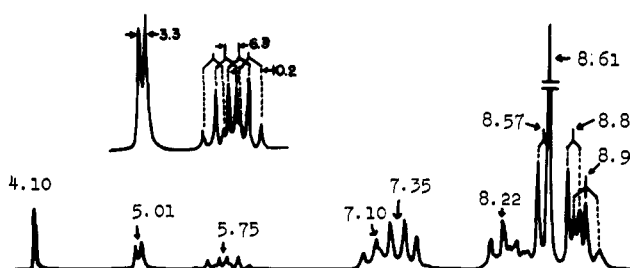Fig. 1.—N.m.r. spectrum of costatolide in chloroform. Positions in τ-units.

Fig. 2.—N.m.r. spectrum of dihydrocostatolide in deuteriochloroform. Positions in τ-units.
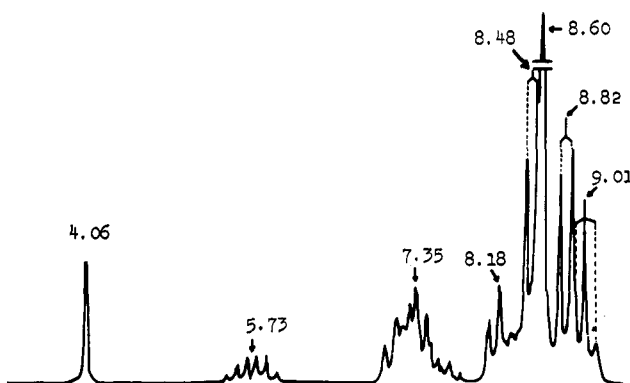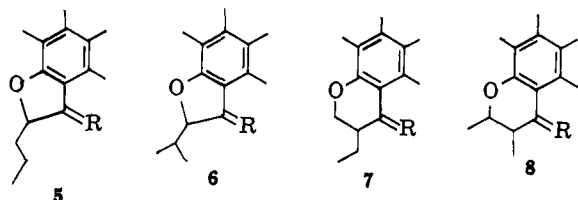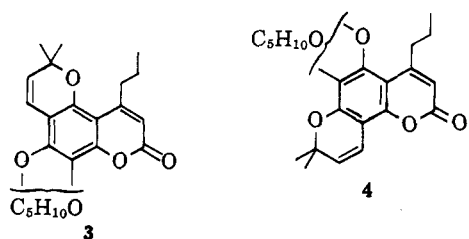

Fig. 3.—N.m.r. spectrum of oxodihydrocostatolide in chloroform. Positions in τ-units.

methylene adjacent to the ring, while the terminal methyl is also visible as a triplet at τ 8.94. The intervening methylene cannot be resolved from the general mass of saturated proton peaks.[18]

On the basis of this evidence, an intermediate structure for costatolide could be written as 3 or 4, with the remaining atoms to be disposed in an additional heterocycle.

The unlocated oxygen atom in 3 (4) is present as a secondary alcohol, identified by its ready oxidation to a ketone in oxodihydrocostatolide. The marked change in the ultraviolet spectrum which accompanies this oxidation, as well as the ready hydrogenolysis of the alcohol function in the formation of deoxydihydrocostatolide, indicates that the alcohol must be benzylic. The proton attached to the carbon bearing the hydroxyl group is visible in the n.m.r. spectra of costatolide and dihydrocostatolide as a doublet at τ 5.0, which vanishes on oxidation (Fig. 3). The appearance of the signal as a doublet suggests that it arises from a proton coupled to only a single neighbor and thus that only the four-part structures, 5a–8a, were possible

a, R = H, OH
b, R = O

(12) A. M. Duffield and P. R. Jefferies, *Australian J. Chem.*, **16**, 123 (1963).

(13) S. Wawzonek, "Heterocyclic Compounds," Vol. 2, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1951, p. 329.

(14) J. A. Lamberton and J. R. Price, *Australian J. Chem.*, **6**, 66 (1953).

(15) J. Polonsky and Z. Baskevitch, *Bull. soc. chim. France*, 929 (1957).

(16) S. S. Dharmatti, G. Govil, G. R. Kanekar, C. L. Khetropal, and Y. P. Virmani, *Proc. Indian Acad. Sci.*, **56A**, 71 (1962).

(17) *Cf.*, for example, C. Djerassi, E. J. Eisenbraun, R. A. Finnegan, and B. Gilbert, *J. Am. Chem. Soc.*, **25**, 2164 (1960).

(18) The model compound 4-n-propyl-5,7-dihydroxycoumarin shows corresponding peaks (in pyridine) at τ 3.99 (C-3), 6.99 (α-methylene), and 9.10 (γ-methyl).
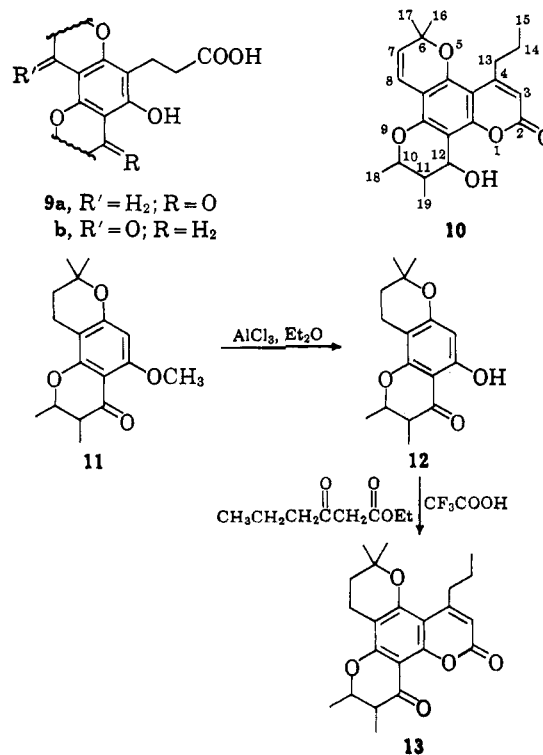
for costatolide. All but one of these could be eliminated on the basis of the n.m.r. spectra of costatolide and its derivatives. These show in each case a multiplet of at least six or seven lines and an intensity of one proton at the position expected for the hydrogen on the carbon to which the ring oxygen is joined (τ 5.67–5.75). The fact that neither the multiplicity nor the position of the signal changes significantly when the secondary alcohol is oxidized to the ketone shows that the proton is not adjacent to the site of this oxidation, and thus that structures 5a and 6a may be rejected. The presence of only one such proton and the high multiplicity of its signal eliminates 7a as well, leaving only 8a.

Confirmation of this choice can be found in the identification of two doublets (τ 8.57 and 8.83 in dihydrocostatolide) as belonging to two methyl groups, each split by a single proton. With this addition, every visible peak in the n.m.r. spectrum of costatolide and its derivatives can be accounted for. The infrared spectrum of oxodihydrocostatolide is also consistent with the part structure 8b. The carbonyl band at 5.89 μ, although at a surprisingly short wave length for an acetophenone with the carbonyl group held near the plane of the ring, is in good agreement with that reported[6] for inophyllolide (14) and significantly different from that of 2-isopropyl-5-methylcoumaranone (5.81 μ), which contains the system 6b.

Once the identities of the various fragments were known, there remained to be determined the relative orientation of the rings, i.e., whether costatolide corresponded to 3 or 4. An attempt was made to decide this question by the hydrogenation of the coumarin ring of oxodihydrocostatolide in the presence of base, a procedure reported to give the phenolic dihydro acid (cf. 9).[19] It was hoped that the relative positions of the phenolic hydroxyl and the keto group could be deduced from the properties of the product. Unfortunately the product of the hydrogenation could not be obtained pure, apparently in part because of a strong tendency to relactonize. The ultraviolet spectrum of the crude product was examined in neutral and basic ethanol, however, and the shift observed on adding base (+45 mμ in the long wave-length band) was strongly suggestive of an o-hydroxy- rather than a p-hydroxyacetophenone system[20] (i.e., 9a rather than 9b).

This observation indicated that structure 13, corresponding to 3, was to be preferred for oxodihydrocostatolide, and thus 10 for costatolide itself.[21]

Synthesis.—In order to confirm the chain of deductions leading to these structural proposals, the synthesis of racemic oxodihydrocostatolide was undertaken. The key intermediate 11 had previously been synthesized by Polonsky[15] in connection with her structural studies on inophyllolide (14, vide infra), and we repeated the synthesis with modifications (see Experimental). The ether 11 was demethylated with aluminum chloride in ether to give the phenol 12. This was condensed with ethyl butyroacetate in the presence of trifluoroacetic acid[22] to give synthetic racemic oxodihydrocostatolide

(19) F. M. Dean, "Naturally Occurring Oxygen Ring Compounds," Butterworths and Co. (Publishers) Ltd., London, 1963, p. 179.
(20) N. A. Valyashko and Y. S. Rozuns, Zh. Obshch. Khim., 17, 755, 783 (1947).
(21) A systematic name for this molecule is 10,11-dihydro-12-hydroxy-6,6,10,11-tetramethyl-4-n-propyl-2H,6H,12H-benzo[1,2-b:3,4-b':5,6-b'']-tripyran-2-one.
(22) L. L. Woods and J. Sapp, J. Org. Chem., 27, 3703 (1962).

9a, R' = H₂; R = O
b, R' = O; R = H₂

10

11 → 12

AlCl₃, Et₂O

$CH_3CH_2CH_2\overset{O}{\overset{||}{C}}CH_2\overset{O}{\overset{||}{C}}OEt$ | CF₃COOH

13

(13) identical in its ultraviolet, infrared, and n.m.r. spectra with material prepared from costatolide.[23] The formation of the coumarin ring, usually the bane of syntheses of highly substituted coumarins,[4] occurred in 33% yield. Attempts to carry out the same reaction in the presence of sulfuric acid, the classical catalyst, gave no product.

The identity of the synthetic and natural materials confirmed the correctness of our deductive sequence and allowed us to add costatolide to the small group of naturally occurring 4-substituted coumarins. Its most obvious relative is inophyllolide (14),[15] isolated from

14　　15

16a, R = n-propyl
b, R = phenyl
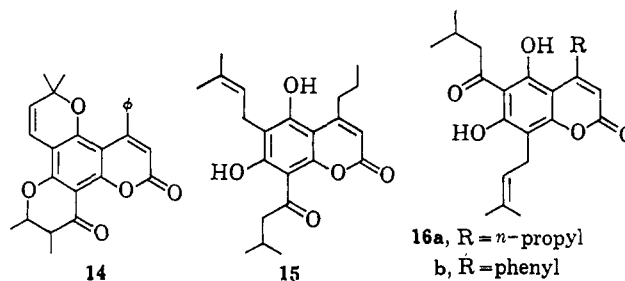
Calophyllum inophyllum L., another member of the same genus. The 4-n-propyl group appears in mammein (15),[17] which is accompanied in *Mammea americana* L. (Guttiferae) by its isomer (16a)[17] and a related coumarin (16b)[24] in which the n-propyl group is replaced by phenyl.

Stereochemistry.—The presence of three asymmetric carbons in costatolide left the determination of their configurations as the final problem to be resolved. This was accomplished by a detailed examination of the n.m.r. coupling constants among the various protons on the 2,3-dimethylchromanol ring.

(23) In addition, the natural and racemic materials melted at the same point and showed no depression on mixture. This phenomenon has also been observed with the closely related calophyllic acid.[6]
(24) R. A. Finnegan, M. P. Morris, and C. Djerassi, J. Org. Chem., 26, 1180 (1961).

If it is assumed that the proton on C-10 is coupled to its adjacent methyl group with the usual constant of $J \sim 6$ c.p.s.,[25,26] examination of the multiplet at τ 5.73 shows that a second coupling, $J = 10$–11 c.p.s., must be present between C-10 and C-11. Such a coupling implies a dihedral angle approaching $180°$[27] and thus a *trans* diaxial relationship between the two protons. At the same time, the doublet at τ 4.98 in costatolide shows that the protons on C-11 and C-12 are coupled with $J = 3.5$ c.p.s. This value is normally consistent with either an axial–equatorial or an equatorial–equatorial arrangement,[28] but, since the C-10–C-11 coupling requires the C-11 proton to be axial, the former must pertain in this case. Thus the substituents at C-11 and C-12 are *cis* and costatolide can be presented with stereochemical detail as **17**.

17   18
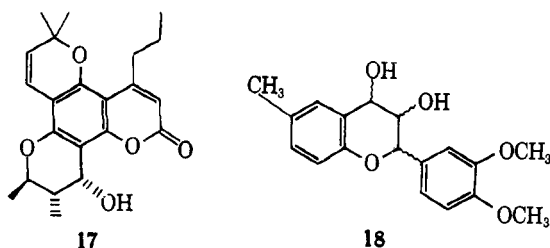
The above arguments are based on the assumption that the atoms 10, 11, and 12, together with their substituents, resemble an undistorted cyclohexane ring sufficiently closely for the customary terminology and coupling constants to be applicable. Fortunately, an excellent model for our chroman system is available in the n.m.r. studies which have been made of a number of epimeric flavan derivatives (**18**).[29] In this system, which would be subject to much the same conformational distortions as the dimethylchromanol ring of costatolide, the coupling constants have been found to be $J_{2(ax),3(eq)} = 0$–1.0 c.p.s., $J_{2(ax),3(ax)} = 7.1$–10 c.p.s., $J_{3(ax),4(ax)} = 5.8$–7.5 c.p.s., $J_{3(ax),4(eq)} = 3.3$–3.5 c.p.s., $J_{3(eq),4(ax)} = 3.9$–4.3 c.p.s., $J_{3(eq),4(eq)} = 0$–1.0 c.p.s. As may be seen, the agreement between our values and those found for the corresponding configurations in the flavan series is excellent.

Confirmation of these configurational assignments was obtained by the reduction of oxodihydrocostatolide with sodium borohydride in an acidic buffer. Two products were obtained, one of them identical with dihydrocostatolide. The other, present in larger amounts, was its epimer, epidihydrocostatolide (**19**). The ultraviolet and n.m.r. spectra of the two compounds are the same except that the C-12 proton signal (τ 5.01, $J = 3.3$ c.p.s., in the natural isomer) appears at τ 5.24 ($J = 7.2$ c.p.s.) in the epimer. Both the increased shielding[30] and the change in coupling are consistent with an equatorial proton in dihydrocostatolide which becomes axial in the epimer.

If the reduction was carried out with sodium borohydride in unbuffered methanol, four products were

(25) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, p. 87.

(26) The observed splittings for the two methyl groups C-18 and C-19 are ca. 5 and 7 c.p.s. The value derived from the high resolution spectrum of dihydrocostatolide is 6.3 c.p.s.

(27) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

(28) R. V. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958).

(29) J. W. Clark-Lewis, L. M. Jackman, and L. R. Williams, *J. Chem. Soc.*, 3858 (1962).

(30) See ref. 25, p. 116.

obtained. Two of these corresponded to those described above; the other two, which showed identical ultraviolet spectra, are presumably the epimeric alcohols in which inversion has also occurred at C-11 (**20a** and **b**).

19   20a, R = H; R' = OH
                      b, R = OH; R' = H
                      c, R, R' = O
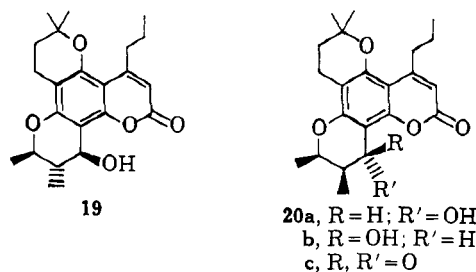
When oxodihydrocostatolide was treated with dilute ethanolic KOH, there were obtained both recovered starting material and a new compound, with the same ultraviolet spectrum, which was taken to be the 10,11-*cis* isomer (**20c**). The n.m.r. spectrum of this material shows the C-10 proton at τ 5.42, $J_{10,11} = ca.$ 3 c.p.s., consistent with such a change. Similarly, the synthesis of oxodihydrocostatolide from the chromatographically homogeneous phenol **13** led to two products. One of these was the desired product; the other was identical in its chromatographic behavior with the epimer produced by equilibration in base.

Inophyllolide (**14**) shows a n.m.r. spectrum which very much resembles that of oxodihydrocostatolide and which suggests that the two have the same configuration. On the other hand, inophyllolide is reported to be optically inactive, while its corresponding coumarinic acid, calophyllic acid, also found in *C. inophyllum*, is apparently known in both enantiomeric forms.[6] Further investigation of the stereochemistry of these 2,3-dimethylchroman derivatives would appear to be called for.

Experimental

All melting points were taken on a Kofler hot-stage microscope (unless stated otherwise) and are corrected. Combustion analyses were performed by Dr. Alfred Bernhardt of Mülheim (Ruhr), West Germany. The infrared spectra were taken on a Perkin-Elmer Model 21 spectrophotometer. The letter in parenthesis signifies a strong, medium, or weak absorption. N.m.r. spectra were obtained by Mr. B. J. Nist on a Varian HR-60 spectrometer. The letter in parentheses refers to the multiplicity with s = singlet, d = doublet, t = triplet, q = quadruplet, and m = multiplet. The number following the letter is the integrated intensity, estimated unless otherwise stated.

Isolation of Costatolide.—From an injured tree of *Calophyllum costatum* (Guttiferae) was obtained a mixture (18.7 g.) of exuded resin and bark. The mixture was digested with several small portions of benzene leaving 3.5 g. of bark and 2.0 g. of white, water-soluble material. The dark red benzene solution, concentrated to approximately 50 ml., deposited on standing 4.62 g. of colorless needles, m.p. 181–182°. A second crop (3.85 g.) after recrystallization from benzene gave 2.96 g., m.p. 181–182°. Yield, based on exuded resin and material from the two crops, was 45%. The crystalline material, costatolide, was soluble in the common organic solvents, gave no coloration with ferric chloride, gave no 2,4-dinitrophenylhydrazone, was not reduced by sodium borohydride, and was insoluble in 10% aqueous sodium hydroxide: infrared (CCl$_4$): 2.75 (w), 5.74 (s), 6.08 (w), 6.18 (m), 6.28 (s) μ; λ_{max}^{EtOH} 230 mμ (log ϵ 4.33), 276 (sh), 285 (4.38), 290 (sh), 304 (sh), 328 (4.12); n.m.r. (CHCl$_3$): τ 8.94 (t, 3), 8.88 (d, 3), 8.59 (d, 3), 8.52 (s, 6), 7.06 (t, 2), 6.85 (broad, 1), 5.67 (m, 1), 4.40 (d, 1), 3.96 (s, 1), 3.24 (d, 1) (Fig. 1); [α]^{25}D −19.9° (c 0.42, CHCl$_3$), [α]^{25}D −50.4° (c 1.55, acetone); mass spectrum (relative intensity), 370 (25.2), 355 (100), 337 (8.8), 299 (31), 271 (9.9).

Anal. Calcd. for C$_{22}$H$_{26}$O$_5$: C, 71.33; H, 7.08; mol. wt.,

370.4. Found: C, 71.43; H, 7.06; mol. wt., 370 (mass spectrometrically).

A red color was produced in solutions of costatolide when they were exposed to sunlight or an ultraviolet source. Color formation was quite solvent dependent and was especially rapid and facile in chloroform.

Dihydrocostatolide.—Costatolide (100 mg., 0.27 mmole), added to 15 mg. of prereduced platinum oxide in 15 ml. of methanol, was stirred 1 hr. in the presence of hydrogen. At the end of this time the sample had taken up 0.267 mmole of hydrogen and reduction had essentially stopped. The platinum was filtered off, and after the methanol was removed by means of a rotary evaporator, the material crystallized. Crystallization from petroleum ether (30–50°) gave 77 mg., m.p. 150–160°, of a highly fluorescent compound. Recrystallization from petroleum ether gave 67 mg. (67%) with m.p. 159–160°; infrared (CCl₄): 2.90 (w), 5.75 (s), 6.16 (m), 6.22 (m) μ; λ_{max}^{EtOH} 215 mμ (log ϵ 4.59), 236 (sh), 251 (3.84), 261 (3.86), 330 (4.16); n.m.r. (CDCl₃): τ 8.98 (t, 3), 8.83 (d, 3), 8.61 (s, 6), 8.57 (d, 3), 8.22 (t, 2), 7.35 (t, 2), 7.10 (t, 2), 5.75 (m, 1), 5.01 (d, 1), 4.10 (s, 1) (Fig. 2); $[\alpha]^{25}$D −26.0° (c 0.40, CHCl₃).

Anal. Calcd. for C₂₂H₂₈O₅: C, 70.94; H, 7.58. Found: C, 70.81; H, 7.49.

Deoxydihydrocostatolide.—To 150 mg. of prereduced platinum oxide in 10 ml. of acetic acid was added 500 mg. (1.35 mmoles) of costatolide in 35 ml. of acetic acid. After the solution had been stirred for 2.5 hr. in the presence of hydrogen, reaction had stopped and 2.97 mmoles of hydrogen had been consumed. The platinum was filtered off and the filtrate was evaporated. The product was crystallized from ice-cold ether giving for the first crop 140 mg. (m.p. 110–122°) and for the second 136 mg. (m.p. 105–120°). The two fractions were combined, and two crystallizations from cold ether gave 31.2 mg. (6.2%) with m.p. 115–120°; infrared (CCl₄): 5.75 (s), 6.23 (s), 6.33 (m) μ; λ_{max}^{EtOH} 213 mμ (log ϵ 4.5), 230 (sh), 254 (3.84), 263 (3.96), 331 (4.17); n.m.r. (CHCl₃): τ 8.98 (t), 8.93 (d), 8.63 (s), 8.57 (d), 8.23 (t), 7.35 (t), 7.08 (t), 5.75 (m), 4.10 (s); $[\alpha]^{24}$D −54° (c 0.192, CHCl₃).

Anal. Calcd. for C₂₂H₂₈O₄: C, 74.13; H, 7.92. Found: C, 74.14; H, 7.83.

Oxodihydrocostatolide.—Dihydrocostatolide (1.466 g., 3.9 mmoles) was dissolved in 50 ml. of purified acetone, and to this solution chromic acid reagent (2.67 M CrO₃ in 20% sulfuric acid) was slowly added until the solution remained reddish orange. The mixture was stirred for 10 min., diluted with water, and extracted with three portions of ether, which were washed with water and dried over anhydrous magnesium sulfate. Removal of the ether left a solid which was crystallized twice from ethanol–water to give 0.980 g. (67%) of ketone: m.p. 163–165°; infrared (CCl₄): 5.76 (s), 5.89 (m), 6.20 (s), 6.32 (s), 6.42 (s) μ; λ_{max}^{EtOH} 221 mμ (log ϵ 4.49), 289 (4.36), 315 (4.22), 340 (sh); n.m.r. (CHCl₃): τ 9.01 (t, 3), 8.82 (d, 3), 8.60 (s, 6), 8.48 (d, 3), 8.18 (t, 2), 7.35 (t, 2), 7.16 (t, 2), 5.73 (m, 1), 4.06 (s, 1) (Fig. 3); $[\alpha]^{22}$D −53.3° (c 0.42, CHCl₃).

Anal. Calcd. for C₂₂H₂₆O₅: C, 71.33; H, 7.08. Found: C, 71.18; H, 7.09.

Base Degradation of Costatolide.—Costatolide (100 mg., 0.27 mmole) was added to 5 ml. of 5% sodium hydroxide solution in a small distilling flask fitted with a nitrogen bubbler. The mixture was heated at 100° for 2 hr. while a slow stream of nitrogen was passed through and then into 2,4-dinitrophenylhydrazine reagent. The precipitate which formed in the reagent (7 mg.) was chromatographed on Whatman No. 1 paper using the organic phase of cyclohexane–methanol–acetic acid–water (60:12:1:2) and showed two spots, R_f 0.69 and 0.54. These were identified as the dinitrophenylhydrazones of acetone and acetaldehyde by simultaneous comparison with authentic samples (R_f 0.69 and 0.56, respectively).

Base Degradation of Deoxydihydrocostatolide.—A mixture of deoxydihydrocostatolide (70 mg., 0.197 mmole) and 5 ml. of 50% sodium hydroxide solution were heated in an oil bath at 200° for 5 hr. while a slow stream of nitrogen was passed through. The volatile products were condensed from the gas stream in a Dry Ice–alcohol trap. The distilled material was analyzed by v.p.c. using a SE-30 silicone column and had a retention time of 3.3 min. at 74°, identical with that of 2-pentanone, but significantly different from those of acetone, ethanol, 3-methyl-2-butanone, or 4-methyl-2-pentanone.

The material was converted to its 2,4-dinitrophenylhydrazone and chromatographed on Whatman No. 1 paper with the solvent

given above. The R_f value was the same (0.73) as that of an authentic sample of 2-pentanone dinitrophenylhydrazone.

Hydrogenation of Oxodihydrocostatolide in Base.—A mixture of 100 mg. (0.27 mmole) of oxodihydrocostatolide, 100 mg. of 5% palladium on charcoal, 20 ml. of 10% potassium hydroxide solution, and 20 ml. of methanol was stirred at room temperature in the presence of hydrogen for 1 week. The catalyst was filtered off and the filtrate was acidified with dilute sulfuric acid and extracted with three portions of ether. The ethereal extract was washed with one portion of water, then extracted with three portions of 5% sodium bicarbonate solution. The sodium bicarbonate extract was washed once with ether, acidified, and extracted with ether. The ethereal extract was washed with three portions of water, dried over anhydrous sodium sulfate, and finally evaporated leaving a light yellow oil. All attempts to obtain a pure product were unsuccessful: λ_{max}^{EtOH} 297 and 333 mμ, $\lambda_{max}^{EtOH-OH^-}$ 299 and 378 mμ.

Senecioyl Chloride.—To senecioic acid (3,3-dimethylacrylic acid, 51.5 g., 0.515 mole) in a 100-ml. round-bottom flask fitted with a drying tube was added purified thionyl chloride (67 g., 0.568 mole). The reaction, which was initially endothermic, was allowed to warm to room temperature. After standing at room temperature for 24 hr., the product was distilled at 18 mm. The fraction boiling at 47–49° was collected to give 58.2 g. (95.5%) of senecioyl chloride.

Tigloyl Chloride.[31]—Tiglic acid (8 g., 0.08 mole) in a small pear-shaped flask fitted with a reflux condenser was allowed to react with phosphorus trichloride (3.82 ml., 6 g., 0.0438 mole) at 77° for 2 hr. After cooling to room temperature, the upper layer was separated from the lower sirupy layer and distilled at 12 mm. The fraction boiling between 42–43° was collected giving 8.7 g. (92%) of tigloyl chloride: infrared (CCl₄): 5.70 and 6.06 μ.

5,7-Dihydroxy-2,2-dimethylchromanone.—To a clean, dry, 3-l., three-necked flask fitted with a stirrer, nitrogen inlet tube, and a dropping funnel were added freshly distilled nitrobenzene (1320 ml.), anhydrous phloroglucinol (100 g., 0.894 mole), and aluminum chloride (94 g., 0.70 mole). The mixture warmed slightly and was cooled to room temperature. Senecioyl chloride (80 g., 0.678 mole) was then added dropwise over 2.5 hr. at such a rate as to maintain the temperature at about 30°. The mixture was stirred at room temperature for 4 days, and the dark brown solution then poured into a mixture of 1000 g. of ice and 650 ml. of dilute hydrochloric acid. After being warmed on the steam bath for 1 hr. the mixture was extracted with ether. The ethereal extract was washed with three portions of water then steam distilled to remove the ether and nitrobenzene. After all of the nitrobenzene had been removed, the hot aqueous liquid (about 2 l.) deposited crystals on cooling. Collection of the colorless needle-like crystals by filtration and drying at 80° under reduced pressure gave 59.55 g. (61.8%) of the desired compound. Recrystallization from alcohol–water gave m.p. 194–201° (lit.[32] m.p. 198°).

5-Hydroxy-7-methoxy-2,2-dimethylchromanone.—Methyl iodide (34 g., 0.24 mole) was added to a stirred mixture of 5,7-dihydroxy-2,2-dimethylchromanone (59.5 g., 0.28 mole), acetone (2500 ml.), and potassium carbonate (119 g.). The mixture was refluxed with stirring for 1.5 hr. after which time more (14.9 ml.) methyl iodide was added along with 60 g. of potassium carbonate. After refluxing an additional 1.5 hr., the mixture was cooled to room temperature, and the potassium carbonate was filtered off. Approximately three-fourths of the acetone was then evaporated and a large volume of water was added. The mixture was extracted with three portions of ether, which were combined, washed with three portions of water, and dried over anhydrous sodium sulfate. Removal of the ether left an oil which was crystallized from alcohol. Recrystallization from alcohol–water gave 41.9 g. (67%) after drying under reduced pressure. The compound gave m.p. 61–64° (lit.[31] m.p. 65–66°); infrared (neat): 6.07 (s) and 6.32 (m) μ; n.m.r. (CCl₄): τ 8.60 (s, 6), 7.50 (s, 2), 6.37 (s, 3), 4.3 (pair of doublets), −1.43 (s, 1).

7-Methoxy-5-hydroxy-2,2-dimethylchroman.—7-Methoxy-5-hydroxy-2,2-dimethylchromanone (41.9 g., 0.143 mole) was added to a 3-l., three-necked flask fitted with a Vibro Mischer, along with 420 ml. of alcohol, 800 ml. of water, 208 ml. of glacial acetic acid, 1660 g. of amalgamated mossy zinc, and 400 ml. of concentrated hydrochloric acid. The mixture was stirred at room temperature for 3 hr., after which time the zinc was filtered off.

(31) C. Barger, W. F. Martin, and W. Mitchell, *J. Chem. Soc.*, 1820 (1937).

(32) W. Bridge, R. G. Hayes, and A. Robertson, *ibid.*, 279 (1937).

After the addition of water to the filtrate, it was extracted with three portions of ether. The ethereal extract was washed with three portions of 1% sodium bicarbonate solution and three portions of water, and finally dried over anhydrous magnesium sulfate. Removal of the ether left 34.8 g. (88%) of a light-colored oil: infrared (neat): 2.94 (s), 6.14 (s), 6.27 (s), 6.97 (s) μ; n.m.r. (CCl$_4$): τ 8.77 (s, 6), 8.34 (t, 2), 7.52 (t, 2), 6.46 (s, 3), 4.43 (s, 2), 3.76 (s, 1), integrated areas.

Tiglate of 7-Methoxy-5-hydroxy-2,2-dimethylchroman.[15]—To a 1-l., one-necked flask were added 7-methoxy-5-hydroxy-2,2-dimethylchroman (34.8 g., 0.167 mole), 300 ml. of pyridine, and tigloyl chloride (35.9 g., 0.303 mole). After approximately 1 hr. at room temperature crystals began to form. The reaction was allowed to proceed at room temperature for 18 hr., after which time more tigloyl chloride (3 g., 0.0253 mole) and 50 ml. of benzene were added. The reaction mixture was then kept at 32° for 6 hr. The dark reaction mixture was poured into a large amount of crushed ice and dilute hydrochloric acid and extracted with ether. The ethereal extract was washed several times with dilute hydrochloric acid (until the washings were acidic), then with three portions of water, and finally with three portions of dilute sodium hydroxide solution. After drying over anhydrous magnesium sulfate, the ether was removed leaving 23.1 g. (48%) of the desired compound as a reddish viscous oil: infrared (neat): 5.76 (s), 6.05 (w), 6.11 (s), 6.30 (s), 6.67 (s) μ; n.m.r. (CCl$_4$): τ 8.74 (s, 6), 8.3 (t, 2), 8.17 (d, 3), 8.11 (s, 3), 7.61 (t, 2), 6.34 (s, 3), 3.92 (s, 2), 3.0 (m, 1).

2,3,8,8-Tetramethyl-4-oxo-5-methoxy-9H,10H-benzo[1,2-b:3,4-b']dipyran (11).[15]—Freshly distilled nitrobenzene (107 ml.) and aluminum chloride (22.8 g., 0.171 mole) were added to the tiglate ester of 7-methoxy-5-hydroxy-2,2-dimethylchroman (23.1 g., 0.080 mole) in a 250-ml. flask. The mixture was allowed to react at room temperature for 72 hr., after which time it was poured into crushed ice and dilute hydrochloric acid. The product was extracted with three portions of ether and the ethereal extract was washed with water, three 25-ml. portions of 10% sodium hydroxide solution, and finally with water until the water extracts were neutral. Removal of the ether and nitrobenzene by steam distillation left a dark oil which was dissolved in benzene and treated with a small amount of Norit. Crystallization from ether–petroleum ether gave 3.9 g. (16.7%), m.p. 155–159° (lit.[15] m.p. 160–161°), of the desired product: infrared (CCl$_4$): 5.94 (m), 6.19 (s), 6.30 (s) μ; n.m.r. (CCl$_4$): τ 8.90 (d, 3), 8.71 (s, 6), 8.57 (d, 3), 8.28 (t, 2), 7.49 (t, 2), 6.28 (s 3), 4.76 (m, 1), 4.21 (s, 1).

2,3,8,8-Tetramethyl-4-oxo-5-hydroxy-9H,10H-benzo[1,2-b:3,4-b']dipyran (12).—The previous methyl ether (3.9 g., 0.0134 mole), dissolved in 131 ml. of anhydrous ether, was allowed to react with aluminum chloride (7.82 g., 0.0558 mole) at reflux temperature for 16 hr., after which time the reaction mixture was poured into ice and dilute hydrochloric acid. The product was extracted with four portions of ether and the ethereal extract was washed with three portions of water before being dried over anhydrous magnesium sulfate. Removal of the ether left crystals which were recrystallized from methanol giving 1.9 g. (51%), m.p. 127–129°, of the desired phenol: λ_{max}^{EtOH} 216 mμ (ϵ 22,900), 293 (20,800), 330 (3100, sh); $\lambda_{max}^{EtOH-OH^-}$ 240 mμ (ϵ 12,300, sh), 292 (17,700), 365 (4100); infrared (CCl$_4$): 6.07 (s), 6.15 (w), 6.28 (w), 6.74 (m) μ; n.m.r. (CCl$_4$): τ 8.85 (d, 3), 8.6 (s, 6), 8.52 (d, 3), 8.28 (t, 2), 7.47 (t, 2), 6.02 (m, 1), 4.20 (s, 1), −1.20 (s, 1).

Anal. Calcd. for C$_{16}$H$_{20}$O$_4$: C, 69.54; H, 7.30. Found: C, 69.25; H, 7.04.

Synthetic Oxodihydrocostatolide (13).—To the previously prepared phenol (100 mg., 0.362 mmole) in a small pear-shaped flask fitted with a reflux condenser were added ethyl butyroacetate (114 mg., 0.724 mmole) and 10 ml. of trifluoroacetic acid. The solution was refixed for 24 hr., poured into water, and extracted with three portions of ether. The ethereal extract was washed with three portions of water, then dried over anhydrous magnesium sulfate. Removal of the ether left an oil which was purified by preparative t.l.c. (66% ether, 33% petroleum ether, and 1% acetic acid). The t.l.c. plates showed mainly two spots (by ultraviolet) which had the same R_f values as natural and epimeric oxodihydrocostatolide. The "upper spot" (which corresponded to the natural isomer) gave 33.3 mg. (24.6%), m.p. 163–165°. A mixture melting point with natural oxodihydrocostatolide occurred at 163–166°; infrared (CCl$_4$): 5.76 (s), 5.89 (m), 6.20 (s), 6.32 (s), 6.42 (s) μ; λ_{max}^{EtOH} 221 mμ (log ϵ 4.49), 289 (4.36), 315 (4.22), 340 (sh); $\lambda_{max}^{EtOH-OH^-}$ 212, 240 (sh), 270 (sh), 333, 383 mμ;

n.m.r. (CHCl$_3$): τ 9.01 (t, 3), 8.82 (d, 3), 8.60 (s, 6), 8.48 (d, 3), 5.73 (m, 1), 4.06 (s, 1). The infrared spectra of natural and synthetic oxodihydrocostatolide were identical.

Anal. Calcd. for C$_{22}$H$_{26}$O$_5$: C, 71.33; H, 7.08. Found: C, 71.30; H, 7.32.

The "lower spot" gave 11.0 mg. (8.9%) of an oil with λ_{max}^{EtOH} 221, 289, 315, and 340 (sh) mμ.

Reduction of Oxodihydrocostatolide with Sodium Borohydride.—To oxodihydrocostatolide (100 mg., 0.27 mmole) dissolved in 20 ml. of methanol were added water (1 ml.) and sodium borohydride (100 mg.). After 15 min. at room temperature the mixture was made slightly acidic with dilute hydrochloric acid. Water was added and the material was extracted with ether. The ethereal extract was washed with water, then dried over anhydrous magnesium sulfate. Removal of the ether left an oil which, on t.l.c., gave four intensely blue fluorescent spots. These were labeled, from the most polar to the least polar, A, B, C, and D, respectively. The spot corresponding to compound B had the same R_f value as dihydrocostatolide. All four compounds, when separated by preparative t.l.c., showed ultraviolet spectra which were identical with that of dihydrocostatolide. The yields of the various fractions were A = 38.3 mg., B = 14.7 mg., C = 1.7 mg., and D = 10.3 mg.

Another experiment under the same conditions using 200 mg. of oxodihydrocostatolide gave sufficient material for an optical rotation of A and D and also an n.m.r. spectrum of compound A: A, [α]^{25}D −42.7° (c 0.32, CHCl$_3$), and D, [α]^{25}D −28.8° (c 0.175, CHCl$_3$); the n.m.r. spectrum of A was identical with that of dihydrocostatolide except that the doublet at τ 5.01 (J = 3.3 c.p.s.) had shifted to 5.24 (J = 7.2 c.p.s.).

Reduction of Oxodihydrocostatolide with Sodium Borohydride (Acidic Conditions).—Oxodihydrocostatolide (10 mg., 2.7 × 10^{-5} mole) in 2 ml. of methanol was added to a mixture of 5 ml. of methanol, 5 ml. of water, 0.5 ml. of 10% sodium hydroxide solution, and approximately 100 mg. of potassium biphthalate (the solution was slightly acidic). Sodium borohydride (100 mg.) was added to this solution. After 5 min. at room temperature dilute hydrochloric acid was added to decompose the excess borohydride. The solution was diluted with water and extracted with ether; the ethereal extract was washed with water. After drying over anhydrous sodium sulfate, the ether was removed leaving an oil which gave by t.l.c. essentially two spots identical with spots A and B of the previous experiment. Compound A was predominant.

Equilibration of Oxodihydrocostatolide with Base.—Oxidihydrocostatolide (100 mg., 0.27 mmole) was dissolved in 20 ml. of ethanol and to this solution was added 0.128 ml. of 10% sodium hydroxide solution (0.32 mmole). The solution turned yellow and was allowed to stand at room temperature for 30 min. before being acidified with dilute hydrochloric acid. The solution was extracted with two portions of ether and the ethereal extract was washed with three portions of water, then dried over anhydrous magnesium sulfate. Removal of the ether left an oil which was separated by preparative t.l.c. The material which had the same R_f value as natural oxodihydrocostatolide, as well as the same ultraviolet spectrum, amounted to 9.3 mg. and gave m.p. 161–165° (m.m.p. 161–165°).

Another fraction (5.6 mg.) was collected which was more polar than natural oxodihydrocostatolide. The ultraviolet spectrum of the more polar material was identical with that of the natural isomer; n.m.r. (CDCl$_3$): τ 9.01 (t, 3), 8.90 (d, 3), 8.72 (d, 3), 8.60 (d, 6), 8.20 (d, 2), 7.35 (t, 2), 7.16 (t, 2), 5.42 (m, 1), 4.15 (s, 1).

The remaining material on the t.l.c. plates was collected to give 72.3 mg. which showed a positive ferric chloride test. The material was dissolved in 10 ml. of benzene and treated with a trace of p-toluenesulfonic acid. Benzene was slowly distilled off for 1 hr.; water was then added and the mixture was extracted with three portions of ether and dried over anhydrous magnesium sulfate. Removal of the ether left an oil which was chromatographed as above giving 9.1 mg. of the natural isomer and 23.7 mg. of the epimer.