Sesquiterpene Lactones of *Eupatorium serotinum*

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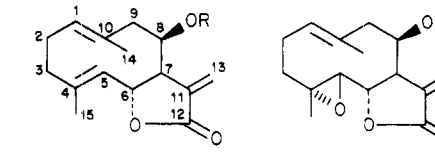
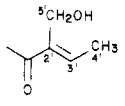
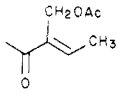
The isolation of four new germacranolides from *Eupatorium serotinum* Michx. is reported. Structures and stereochemistry of **1a,b** and **2a** were established by NMR spectrometry and chemical interconversions. The structure of euserotin (**3**), an unusual germacradienecarboxylic acid, was elucidated by X-ray crystallography. The flavones pectolinarigenin and hispidulin were also found.

In the course of our study[3-5] of *Eupatorium* species *sensu stricto* which elaborate a number of sesquiterpene lactones with cytotoxic and antitumor activity[6] we have examined *E. serotinum* Michx. and hereby report isolation and structure determination of four new germacranolides **1a,b**, **2a**, and **3**. Pectolinarigenin (5,7-dihydroxy-4',6-dimethoxyflavone) and hispidulin (6-methoxy-4',5,7-trihydroxyflavone) were also found.



1a, R = 

b, R = 

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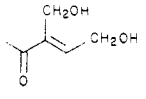
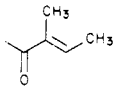
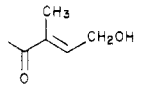
d, R = 

e, R = H

2a, R = 

b, R = 

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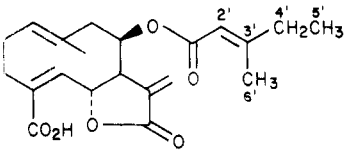

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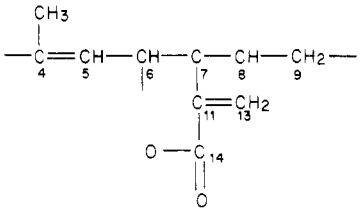
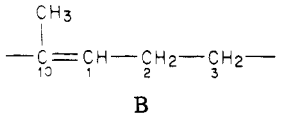
We commence with **1a** (mp 110 °C, $C_{20}H_{26}O_6$) and **1b** (mp 162–163 °C, $C_{22}H_{28}O_6$) whose structures were elucidated relatively easily by spectrometric techniques. In each case, spin-decoupling experiments which will not be described in detail (Table I) established the presence of partial structure A (numbering as in complete molecule) where the typical α-methylene-γ-lactone ring was closed



A



B

to either C-6 or C-8 and an ester side chain was attached to the second of these carbon atoms. The ester side chains were derived from α,β-unsaturated acids (^{13}C-carbonyl singlets at 166.09 and 165.16 ppm, respectively—see Table II), $C_5H_8O_3$ in the case of **1a** and $C_7H_{10}O_4$ in the case of **1b** (high-resolution mass spectra—see Experimental Section), and were identified as *cis*-sarracenoyl (in **1a**) and *cis*-acetylsarracenoyl residues (in **1b**) by inspection and decoupling of the ^{1}H NMR spectra (Table I.)[7,8] As expected on this basis acetylation of **1a** afforded a monoacetate identical in all respects with **1b**.

The remaining five carbon atoms of **1a,b** which were represented in the ^{13}C NMR spectrum by one quartet and two triplets in the aliphatic region, one vinyl doublet, and one vinyl singlet had to be combined as shown in partial structure B since neither H-9a nor H-9b was spin coupled to an additional vicinal proton. For the same reason C-10 of B had to be linked to C-9 of A and C-3 of B to C-4 of A, thus yielding the carbon skeleton of a germacradienolide. That the two substances were not heliangolides but *trans*-1(10),*trans*-4-germacradienolides was indicated by the magnitude of $J_{7,13}$ (>3 Hz) and the absence of NOE's involving H-1, H-5, and the vinyl methyls. Lastly the coincidence of chemical shifts and coupling constants in the NMR spectra with those exhibited by authentic *trans,trans*-germacradienolides functionalized at C-6 and C-8, particularly shifts and coupling constants involving H-5, H-6, H-7, and H-8, clearly showed that the lactone ring was fused toward C-6 and trans and that H-8 was cis to H-7.[10] Final proof of this deduction was obtained by hydrolysis of **1a** to a substance similar in all respects (NMR spectrum, rotation) to eupatolide (**1e**) of known relative and absolute configuration.[11-13] Comparison of the NMR spectra of **1a** and **1e** showed that the hydrolysis had occurred without reorientation of the lactone.

(1) Work at The Florida State University was supported in part by a grant (CA-13121) from the U.S. Public Health Service through the National Cancer Institute.

(2) Recipient of a grant from the Belgian Commission for Educational Exchange with the U.S.A. and a Fulbright-Hayes Travel Award.

(3) W. Herz, R. de Groote, R. Murari, and J. F. Blount, *J. Org. Chem.*, **43**, 3559 (1978), which contains references to our earlier work and that of others.

(4) W. Herz, G. Ramakrishnan, and R. Murari, *Phytochemistry*, **17**, 1953 (1978).

(5) W. Herz, R. Murari, and S. Govindan, *Phytochemistry*, **18**, in press.

(6) Most recently, eurecurvin from *E. recurvans* has exhibited presumptive antileukemic activity in the antitumor screen of the NCI.

(7) W. Herz and I. Wahlberg, *J. Org. Chem.*, **38**, 2485 (1973).

(8) W. Herz, J. Poplawski, and R. P. Sharma, *J. Org. Chem.*, **40**, 199 (1975).

(9) In heliangolides, $J_{7,13}$ is smaller than 3 Hz.[7]

(10) Compare with epitulipinolide (**1c**) and its C-8 epimer tulipinolide and the NMR data reported[12] for eupatoriopicrin (**1d**).

(11) R. W. Doskotch and F. S. El-Feraly, *J. Org. Chem.*, **35**, 1928 (1970).

(12) B. Drozdz, H. Grabarcyk, Z. Samek, M. Holub, V. Herout, and F. Šorm, *Collect. Czech. Chem. Commun.*, **37**, 1546 (1972).

(13) A. T. McPhail and K. D. Onan, *J. Chem. Soc., Perkin Trans. 2*, 1798 (1975).

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Table I. ^{1}H NMR Spectra of *E. serotinum* Constituents[a]

	1a	1b[b]	2a	3	3[c]
H-1	4.88 dd, br (10, 4, 1.5)	4.90 dd, br	5.31 dd, br (11, 2)	5.00 dd, br	5.09 dd, br (11, 4, 1)
H-2a	2.3 m	2.3 m	2.4 m	2.3 m	2.65 dq (3, 12)
H-2b	2.25 m	2.25 m	2.2 m	2.0 m	2.25 m
H-3a	2.1 m	2.1 m	2.17 m	3.06 m	3.22 m
H-3b	2.0 m	1.98 m	1.38 m	1.98 m	2.04 m
H-5	4.79 d, br (10, 1)	4.79 d, br	2.86 d (9.5)	5.47 d (10)	5.64 d (10)
H-6	5.16 dd (10, 9)	5.15 dd	4.44 dd (9.5, 9)	5.84 dd (10, 9)	6.54 dd (10, 9)
H-7	2.94 m	2.94 m	3.16 m	2.83 m	3.22 m
H-8	5.81 dd, br (5.5, 2, 1)	5.82 dd, br	5.73 dd, br (5, 1.5, 1)	5.78 m	6.04 dd, br (5.5, 2, 1)
H-9a	2.87 dd, (15, 5.5)	2.87 dd	2.80 dd (15, 5)	2.83 m	2.97 dd (15, 5.5)
H-9b	2.37 dd (15, 2)	2.35 dd	2.36 dd (15, 1.5)	2.33 m	2.44 dd (15, 2)
H-13a	6.28 d (3.5)	6.29 d	6.36 d (3.2)	6.33 d (3.5)	6.44 d (3.5)
H-13b	5.59 d (3)	5.59 d	5.73 d (3)	5.64 d (3)	5.81 d (3)
H-14[d]	1.46 br (1.5)	1.47 br	1.73 br (1)	1.33 br (1)	1.62 d (1)
H-15[d]	1.76 br (1)	1.77 br	1.39		
H-2′				5.57 br (2)	5.67 q (2)
H-3′	6.86 q, br (7)	7.12	6.78		
H-4′	1.93 d (7)[d]	1.97[d]	1.79[d]	2.15 m[e]	1.88 q (7)[e]
H-5′	4.34 br	4.82 br	1.79[d]	1.06 t (7)[d]	0.74 t (7)[d]
H-6[d]				2.15 br	2.18 br
Ac[d]		1.99			

[a] Run at 270 MHz in $CDCl_3$ with Me_4Si as an internal standard. Chemical shifts are in parts per million; figures in parentheses are coupling constants in hertz. [b] Coupling constants identical with those of **1a**. [c] In Py-d_5. [d] Intensity three protons. [e] Intensity two protons.

Table II. ^{13}C NMR Spectra of *E. serotinum* Constituents[a]

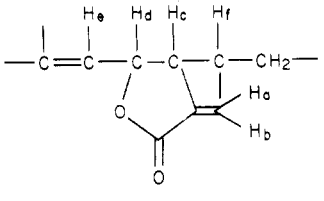
	1a	1b	2a	2b[b]	3[c]
C-1	127.30 d	127.30 d	128.75 d	128.5 d	128.91 d
C-2	26.17 t	26.23 t	24.26 t	23.9 t	27.45 t
C-3	39.36 t	39.41 t	35.90 t	35.6 t	35.89 t
C-4	142.23	142.45	61.85	61.8	131.17
C-5	130.59 d	130.66 d	66.62 d	66.2 d	144.52 d
C-6	75.66 d	75.61 d	75.69 d	75.6 d	69.32 d
C-7	52.68 d	52.76 d	49.68 d	49.1 d	52.73 d
C-8	72.01 d	72.15 d	73.98 d	74.0 d	74.98 d
C-9	42.90 t	43.98 t	43.96 t	43.5 t	43.96 t
C-10	134.11	134.20	131.69	131.2	138.25
C-11	136.67	136.65	136.26	136.0	135.53
C-12	169.55	169.32	168.57	168.5	169.17
C-13	120.98 t	120.99 t	122.57 t	122.3 t	121.75 t
C-14	18.95 q	18.93 q	19.61 q	19.4 q	18.92 q
C-15	17.40 q	17.46 q	17.21 q	16.9 q	170.21
C-1′	166.09	165.16	166.44	165.9	165.54
C-2′	131.93	127.52	127.90 d	126.7 d	113.52 d
C-3′	141.47 d	145.91 d	138.74 d	142.6 d	164.54
C-4′	14.41 q	14.73 q	14.54 q	59.1 t	33.99 t
C-5′	56.49 t	57.33 t	12.17 q	12.4 q	12.01 q
C-1″		170.58			
C-2″		20.67 q			

[a] Run in $CDCl_3$ at 67.9 MHz on a Bruker HX-270 instrument. Values are in parts per million. Unmarked signals are singlets. [b] Taken from ref 13 and revised. [c] Probable assignments, not established by spin decoupling.

Spin-decoupling experiments on the third new lactone, mp 134–5 °C, $C_{20}H_{26}O_5$, showed that partial structure A was modified with C-5 carrying an ether oxygen (upfield shift of H-5 to 2.86 ppm and of H-6 to 4.44 ppm). The other terminus of the ether linkage was C-4 because in the ^{1}H NMR spectrum the broadened methyl signal near 1.8 ppm was replaced by a sharp methyl singlet at 1.39 ppm and as partial structure B was also present. The ester side chain was that of tiglic acid (NMR and mass spectra). Consequently formula **2a** could be written for the new substance. The assigned stereochemistry was based on the same arguments previously[14] used for eupassopilin (**2b**) with whose ^{1}H and ^{13}C NMR spectra the spectra of **2a** are coincident if allowance is made for the difference in ester side chain.[15] In confirmation, hydrolysis of **2a** which was not accompanied by reorientation of the lactone (NMR spectrum) afforded a substance apparently identical with desacetyllipiferolide (**2d**)[18] of established relative and absolute stereochemistry.

Structure elucidation of the fourth and most polar new compound, mp 172–173 °C, $C_{21}H_{26}O_6$, which we named euserotin, was more difficult. The six oxygen atoms of the empirical formula could be accounted for by a carboxylic acid function (IR absorption at 3000–3500 cm^{-1}), the usual α-methylene-γ-lactone grouping (NMR spectrum), and an α,β-unsaturated six-carbon ester side chain (mass spectrum) which was identified as (*E*)-3-methyl-2-hexenoate by analysis of the ^{1}H NMR and ^{13}C NMR spectra. In particular it was noted that the ^{13}C NMR spectrum contained no methyl quartet downfield from ~19 ppm and no methylene triplet upfield from 27.5 ppm; consequently the double bond was *E* rather than *Z*.

Further analysis of the NMR spectra was dogged by superposition of signals, although spin decoupling in pyridine-d_5 yielded partial structure C where H_f was



C

probably located on the carbon also carrying the ester side chain. Since H_e, unlike H-5 of **1a,b**, did not exhibit allylic

(14) W. Herz and R. P. Sharma, *J. Org. Chem.*, **41**, 1015 (1976).

(15) Table II contains a corrected version of the ^{13}C NMR spectrum of **2b**. As the result of discussions with Professor N. S. Bhacca of Louisiana State University, assignments of C-2 and C-3, C-11 and C-12, and C-14 and C-15 have been interchanged. Similarly in the ^{13}C NMR spectra of euperfolin and euperfolitin,[16] the assignments of C-3 and C-5 should be interchanged. By analogy, values can now be assigned for C-2 and C-9 of lipiferolide (**2d**), peroxyferolide, and their derivatives and correspond to the values of Table II of ref 17, if C-2 and C-9 are interchanged.

(16) W. Herz, P. S. Kalyanaraman, G. Ramakrishnan, and J. F. Blount, *J. Org. Chem.*, **42**, 2264 (1977).

(17) R. W. Doskotch, F. S. El-Feraly, E. H. Fairchild, and C. T. Huang, *J. Org. Chem.*, **42**, 3614 (1977).

(18) R. W. Doskotch, S. L. Keeley, C. D. Hufford, and F. S. El-Feraly, *Phytochemistry*, **14**, 769 (1975).

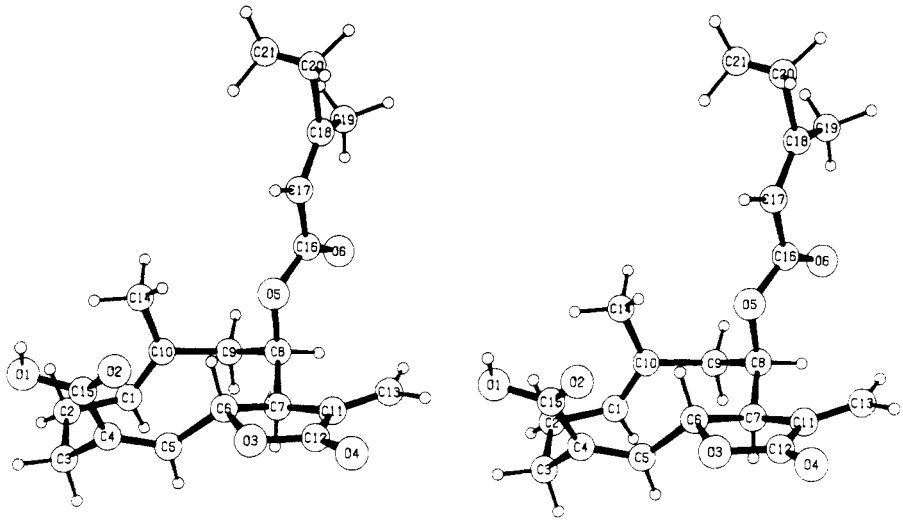


Figure 1. Stereoscopic view of euserotin.

coupling, expansion to gross structure **3** was a biogenetically plausible possibility also in keeping with the ^{13}C NMR spectrum. However, this seemed negated by the chemical shift of H_e (5.47 ppm in $CDCl_3$, 5.64 ppm in C_5D_5N) which appeared too far upfield for location on the β carbon of an α,β-unsaturated acid system. Because of difficulties in reconciling the spectroscopic data with this and other structure proposals,[19] recourse was had to X-ray crystallography.

After some unsuccessful trials, suitable crystals of euserotin were eventually obtained from chloroform–hexane and were found to belong to the relatively rare space group $P2_122_1$, two of whose symmetry operations are 2_1 screw axes which combine twofold rotation with translation. The third operation is a pure twofold rotation axis. Other crystal data are listed in the Experimental Section. As was also inferred from the elementary analysis, the crystal contains solvent which is disordered along the crystallographic twofold axis. Figure 1 is a stereoscopic drawing of the molecule which in view of the known absolute configuration of the congeners **1a,b** and **2a** is also believed to represent the absolute configuration, although it was not possible to deduce this from the X-ray data.

The trans-lactone ring is closed to C-6 and the unsaturated ester side chain which possesses the stereochemistry deduced earlier is attached to C-8 with H-8 α. The molecule is in the crown conformation with the "crossed" arrangement of the two double bonds characteristic of most *trans,trans*-1(10),4-germacradienes and their lactone derivatives and with the methyl and carboxyl group, the latter attached to C-4, projecting above the ring. In the crystal, molecules of euserotin are present as hydrogen-bonded dimers formed by hydrogen bonding between two carboxyl groups across the crystallographic twofold axis. Apparently this does not affect the conformation which is similar to that of more conventional *trans,trans*-germacradienolides;[20-22] in particular the torsion angles C(2)–C(1)–C(10)–C(9) (167°) and C(3)–C(4)–C(5)–C(6) (153°) are not significantly different from those reported for other lactones. Tables III–VII containing bond lengths, bond angles, torsion angles, final atomic parameters, and final anisotropic thermal parameters are available as supplementary material.

C(21) of the ester side chain is at best poorly defined, although it seemed fairly certain that C(19) was the methyl carbon attached to C(18) and that C(20) was the methylene carbon of the ethyl group whose presence has been deduced spectroscopically. To check whether a second carbon was possibly attached to C(19), thus making it the methylene carbon of the ethyl group, we calculated the positions of six hypothetical carbon atoms at C(19). Five of these six atoms made prohibitively short intermolecular contacts (C---C < 3 Å) and the sixth also has a short contact (C---O = 3.15 Å). The closest intermolecular contact for C(21), on the other hand, is 3.35 Å to a C(21) of a molecule related by the crystallographic twofold rotation axis. The C(18)-C(20) and C(20)-C(21) distances in Table V reflect the poor definition of C(20) and especially C(21).

The chemical shift of H-5 (deshielded with respect to H-5 in **1a–d** by 0.7 ppm) is perhaps due to shielding by the 1,10 double bond since the shifts of C-4, C-5, and C-15 are approximately those calculated for an α,β-unsaturated acid with the substitution pattern found in euserotin. The chemical shift of H-1 of germanin A, a germacradienolide of undefined stereochemistry with a carbonyl group attached to C-10,[23] appears to be normal. The unusual six-carbon ester side chain of euserotin seems to have been encountered previously only once as part of a furanoeudesmane from an *Othonna* species.[24]

We conclude with comments on the CD curves of euserotin and its congeners. Compounds **1a,b**, like eupatolide (**1e**),[13] epitulipinolide (**1c**),[11] and its C-8 epimer tulipinolide[11] of established absolute configuration, exhibit negative Cotton effects in the 250-nm region. Consequently the extra presence of the inherently symmetric but asymmetrically perturbed α,β-unsaturated ester chromophore has not affected the sign of the diagnostically valuable α,β-unsaturated lactone band which has been associated with the chirality of the C=C—C=O chromophore.[25] X-ray results have shown that in *trans*-1(10), *trans*-4-germacradienolides with a 6,7-trans ring junction

(19) One of these difficulties was the persistence in the UV spectrum of a shoulder (λ_{max} 265 nm, ϵ 3200), indicative of extended conjugation, on the long-wave side of the band at 217 nm ascribable to superposition of the α,β-unsaturated acid, lactone, and ester chromophores. We are unable to account for the shoulder which may be due to a stubborn impurity or to an electronic interaction of unknown character.

(20) A. T. McPhail and K. D. Onan, *J. Chem. Soc., Perkin Trans. 2*, 1798 (1975), and references therein.

(21) P. J. Cox and G. A. Sim, *J. Chem. Soc., Perkin Trans. 2*, 255 (1977), and references therein.

(22) M. E. Witt and S. F. Watkins, *J. Chem. Soc., Perkin Trans. 2*, 204 (1978).

(23) O. A. Konovalova, K. S. Rybalko, and V. I. Sheichenko, *Khim. Prir. Soedin.*, **10**, 578 (1974).

(24) F. Bohlmann and K.-H. Knoll, *Phytochemistry*, 17, 461 (1978).

(25) A. F. Beecham, *Tetrahedron*, **28**, 5543 (1972).

which exhibit a negative Cotton effect in the 250-nm region[25] the C(13)–C(11)–C(12)–O(4) torsion angle (ω_2) generally is negative, i.e., that the chromophore possesses negative chirality, although it has been suggested recently,[21] in view of the results on alatolide where $\omega_2 = 0$, that the sign of the C(α)–C(β)–C(γ)–O torsion angle (ω_3) may provide a better correlation with the CD.

The values of ω_2 and ω_3 for euserotin are −8.4 and −24.1°, respectively, if it possesses the absolute configuration depicted in Figure 1 and the formulas. The magnitudes are consonant with those deduced for eupatolide,[13] costunolide,[21] and tamaulipin B.[22] On the other hand, its CD curve exhibits a maximum, not a minimum, at 235 nm. Since euserotin contains a second inherently dissymetric chromophore in the form of the α,β-unsaturated acid function and since it would be illogical to postulate the formation of closely related sesquiterpene lactones of opposite absolute configuration in a higher plant, the observed CD curve apparently represents the summation of two separate Cotton effects of opposite sign with other contributions from the first, positive band near 210–220 nm associated with the interacting double bonds in the ten-membered ring[27] and possibly from the asymmetrically perturbed α,β-unsaturated ester.

Substance **2a**, like eupassopin and eupassopilin (**2b**),[16,28] displays a negative Cotton effect near 240 nm as a barely perceptible shoulder on the side of a much stronger negative lower wavelength band which peaks below 210 nm. The shoulder is obscured in the case of desacetyllipiferolide, thus lending substance to the earlier observation[28] that in compounds of this type the substituent on C-8 appears to have some effect on the CD curve. X-ray analyses of two members of this group, eupassopin (eupahyssoppin)[29] and parthenolide[30] (CD curve not reported), yielded negative values for ω_2 (−10, −11.7°) and ω_3 (−24, −21.9°).

Experimental Section

Extraction of *Eupatorium serotinum*. Above-ground parts of *E. serotinum* Michx. (weight 21.2 kg), collected by R. L. Lazor on August 19, 1968, at 3rd and Magnolia, 1 mi East of St. Marks, Wakulla Co., Fla., was extracted with $CHCl_3$ and worked up in the usual manner.[31] The crude gum weighed 200 g. A part (weight 60 g) was absorbed on silicic acid and chromatographed over 750 g of silicic acid (Mallinckrodt 100 mesh) packed in toluene–$CHCl_3$ (4:1). The column was eluted with solvents of increasing polarity, 500-mL fractions being collected in the following order: fractions 1–9, toluene–$CHCl_3$ (4:1); fractions 10–15, toluene–$CHCl_3$ (1:1); fractions 16–22, toluene–$CHCl_3$ (2:3); fractions 23–35, $CHCl_3$; fractions 36–41, $CHCl_3$–MeOH (99:1); fractions 42–50, $CHCl_3$–MeOH (49:1); fractions 51–55, $CHCl_3$–MeOH (24:1); fractions 56–73, $CHCl_3$–MeOH (23:2).

TLC of fractions 16–22 showed the presence of a major constituent (**1b**) which was purified by preparative TLC ($CHCl_3$–MeOH (49:1)) and recrystallized from MeOH: yield 0.25 g; mp 162–163 °C; $[\alpha]^{22}_D$ +90° (*c* 0.14, $CHCl_3$); IR (KBr) 1762, 1735, 1720, 1650, 1265, 995, 850 cm^{-1}; CD curve (deg cm^2/dmol) $[\Theta]_{248}$ −5760 (minimum), $[\Theta]_{232}$ 0, $[\Theta]_{212}$ 307 000 (last reading). The high-resolution mass spectrum displayed only a weak molecular ion (0.1%) but had diagnostic peaks at *m/e* (composition, percent) 230 ($C_{15}H_{18}O_2$, 28.3), 215 ($C_{14}H_{15}O_2$, 7.5), 141 ($C_7H_9O_3$, 46.3), 99 ($C_5H_7O_2$, 46.4), and 81 (C_5H_5O, 100 (base peak)).

Anal. Calcd for $C_{22}H_{28}O_6$: C, 68.02; H, 7.27; O, 24.71; mol wt 388.1884. Found: C, 67.85; H, 7.20; O, 25.27; mol wt (mass spectrum) 388.1909.

Fractions 23–29 deposited a yellow solid on concentration which was recrystallized from MeOH, melted at 215–216 °C (yield 0.25 g), and was identified as pectolinarigenin by comparison with an authentic sample.

Fractions 30–35 contained a major constituent (**2a**) which was purified by preparative TLC ($CHCl_3$–MeOH (97:3)) and recrystallization from MeOH: yield 0.2 g; mp 134–135 °C, $[\alpha]^{22}_D$ −120° (*c* 0.15, $CHCl_3$); CD curve $[\Theta]_{248}$ −860 (sh), $[\Theta]_{210}$ −12 000 (last reading); IR 1765, 1710, 1640, 1250, 1140 cm^{-1}. The high-resolution mass spectrum had a very weak molecular ion (0.1%) but exhibited diagnostic peaks at *m/e* 263 ($C_{15}H_{19}O_4$, 1.2), 246 ($C_{15}H_{18}O_3$, 4.0), 228 ($C_{15}H_{16}O_2$, 8.2), and 83 (C_5H_7O, base peak).

Anal. Calcd for $C_{20}H_{26}O_5$: C, 69.34; H, 7.57; O, 23.09; mol wt 346.1779. Found: C, 69.52; H, 7.49; O, 22.99; mol wt (mass spectrum) 246.1776.

Fractions 42–50 contained a major constituent (**1a**) which was purified by preparative TLC ($CHCl_3$–MeOH (4:1)) and recrystallization from MeOH: yield 0.5 g; mp 110 °C; $[\alpha]^{22}_D$ +47.5° (*c* 0.12, $CHCl_3$); CD curve $[\Theta]_{248}$ −2570 (minimum), $[\Theta]_{227}$ 0, $[\Theta]_{212}$ 18 800 (last reading); IR 3540, 1770, 1715, 1645, 1248 cm^{-1}. The high-resolution mass spectrum had a reasonably strong molecular ion (1.3%) and exhibited diagnostic peaks at *m/e* 246 ($C_{15}H_{18}O_3$, 2.3), 230 ($C_{15}H_{18}O_2$, 46.8), 215 ($C_{14}H_{15}O_2$, 12.8), 99 ($C_5H_7O_2$, base peak), and 81 (C_5H_5O, 17.9).

Anal. Calcd for $C_{20}H_{26}O_5$: mol wt 346.1779. Found: mol wt (mass spectrum) 346.1775.

Acetylation of 50 mg of **1a** (pyridine–acetic anhydride) followed by the usual workup and recrystallization of the crude product from MeOH afforded 48 mg of material, mp 163 °C, identical with **1b**.

Fractions 51–55 gave a yellow solid. Recrystallization from MeOH–$CHCl_3$ afforded 0.3 g of hispidulin, mp 290 °C, identical with an authentic sample in all respects.

Purification of the combined fractions 56–65 by preparative TLC ($CHCl_3$–MeOH) and recrystallization of the major constituent from $CHCl_3$–hexane afforded 0.5 g of **3**: mp 172–173 °C; $[\alpha]^{22}_D$ +75.2° (*c* 0.25, $CHCl_3$); CD curve $[\Theta]_{235}$ +24 000 (maximum), $[\Theta]_{210}$ 14 800 (last reading); UV spectrum λ_{max} 265 and 217 nm (ϵ 3200 and 30 200). Besides the molecular ion (0.9%) the high-resolution mass spectrum exhibited diagnostic peaks at *m/e* 260 ($C_{15}H_{16}O_4$, 1.4), 242 ($C_{15}H_{14}O_3$, 3.6), 114 ($C_6H_{10}O_2$, 4.3), and 97 (C_6H_9O, base peak). The elemental analysis was unsatisfactory due to stubborn retention of solvent which was also revealed by the X-ray analysis.

Anal. Calcd for $C_{21}H_{26}O$: mol wt 347.1728. Found: mol wt (mass spectrum) 374.1729.

Hydrolysis of 1a and 2a. (a) A mixture of 50 mg of **1a** and 40 mg of KOH in 5 mL of water was stirred overnight at room temperature, neutralized with 20% H_2SO_4, saturated with NaCl, and extracted with ethyl acetate. The organic layer was washed with water, dried, and concentrated in vacuo. The residue was chromatographed on a thick-layer plate (silica gel, 6% MeOH in $CHCl_3$). Recrystallization from ethyl acetate–ether furnished 20 mg of **1e**: mp 187–188 °C, $[\alpha]^{22}_D$ +20° (*c* 0.011, acetone); CD curve $[\Theta]_{248}$ −4300 (minimum), $[\Theta]_{232}$ 0, $[\Theta]_{210}$ +32 500 (last reading) (lit.[11] mp 186–188 °C; $[\alpha]$ +29.7°). The NMR spectrum was identical with the reported spectrum and had signals at 6.39 d (2.5, H-13a), 5.58 d (2, H-13b), 5.23 dd (9, 8, H-6), 4.78 d br (40, H-1), 4.75 d (9, H-5), 4.6 dd br (5.5, 2, H-8), 2.80 m (H-7), 2.70 dd (15, 5.5, H-9a), 2.37 dd (15, 2, H-9b), 2.4 m, 2.25 m, 2.0 m, 1.65 m (H-2 and H-3), 1.75 br (H-15), and 1.60 br (H-14) ppm.

(b) A mixture of 80 mg of **2a** and 50 mg of KOH in 5 mL of water was stirred for 24 h at room temperature and worked up as described in the previous paragraph. After recrystallization from ethyl acetate–ether, the product **2d** melted at 178–180 °C; $[\alpha]^{22}_D$ −276° (*c* 0.15, MeOH); CD curve $[\Theta]_{248}$ −650, $[\Theta]_{225}$ −5900, $[\Theta]_{210}$ −12 400 (last reading) (lit.[18] mp 162–163 °C; $[\alpha]$ −295°). Although the melting point was somewhat higher, the NMR spectrum in C_5D_5N was identical with that reported and had signals at 6.47 d (3.5, H-13a), 5.75 d (3, H-13b), 5.30 d br (9, H-1), 5.08 dd (9, 8, H-6), 4.83 m (H-8), 3.28 m (H-7), 3.07 d (9, H-7), 2.70 dd (15, 5.5, H-9a), 2.42 dd (15, 2, H-9b), 2.5 m, 2.15 m (2p),

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(27) M. Suchý, L. Dolejs, V. Herout, F. Šorm, G. Snatzke, and J. Himmelreich, *Collect. Czech. Chem. Commun.*, **34**, 229 (1969).
(28) The values reported in the Experimental Section of ref 14 are correct, but in the paragraph of the Discussion section dealing with the CD curves the words eupassopin and eupassofilin should be interchanged.
(29) K. D. Onan and A. T. McPhail, *J. Chem. Res.*, 12 (5) (1975).
(30) A. Quick and D. Rogers, *J. Chem. Soc., Perkin Trans. 2*, 465 (1975).
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1.20 (H-2 and H-3), 2.07 br (H-14), and 1.44 (H-15) ppm.

X-ray Analysis of *Euserotin*. Crystals of 1 were prepared by slow crystallization from chloroform–hexane. They were orthorhombic, space group $P2_12_12_1$, with a = 7.270 (2) Å, b = 11.113 (4) Å, c = 27.226 (8) Å, and Z = 4. The intensity data were collected on a Hilger-Watts diffractometer (Ni-filtered Cu Ka radiation, θ–2θ scans, pulse height discrinimation). A crystal measuring approximately 0.25 × 0.4 × 0.7 mm was used for data collection. A total of 1733 reflections were measured for θ <57°, of which 1356 were considered to be observed [$I > 2.5\sigma(I)$]. The intensities of the five check reflections declined gradually to 90% of the original values at the end of the 2.5-day period required for data collection. The intensity data were corrected for this decline. At the end of the data collection periods, the two ends (along the a axis) of the crystal had become opaque, with only the center half of the crystal remaining clear.

The structure was solved by a multiple-solution procedure[32] and full-matrix least squares was used for all refinements. Twenty-four nonhydrogen atoms were found on the E map and two more were found on an electron density map on the basis of these atoms. A difference map calculated after isotropic refinement of these 26 atoms (all but C(21)) showed the presence of disordered solvent in the crystal. Three peaks, two of which were located on crystallographic twofold axes, were assigned carbon scattering factors. The refinement was continued with anisotropic thermal parameters for the 46 carbon and oxygen atoms of the molecule of euserotin and isotropic temperature factors for the three "solvent" atoms. C(21) was found on a difference map calculated after this refinement. The positions of all hydrogen atoms were calculated on the basis of the molecular geometry. For the final refinement anisotropic thermal parameters were used for all carbons and oxygens of euserotin except C(21) and isotropic temperature factors were used for C(21), the three "solvent" atoms, and the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are R = 0.089 and R_w = 0.102 for the 1356 observed reflections. The final difference map has no peaks greater than ±0.3 e Å^{-3}.

(32) G. Germain, P. Main, and M. Woolfson, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).

Registry No. **1a**, 70550-00-4; **1b**, 70550-01-5; **1e**, 6750-25-0; **2a**, 70527-88-7; **2b**, 57718-81-7; **2d**, 56245-55-7; **3**, 70527-89-8; pectolinarigenin, 520-12-7; hispidulin, 1447-88-7.

Supplementary Material Available: Tables III–VII listing final atomic parameters, final anisotropic thermal parameters, bond lengths, bond angles, and torsion angles of euserotin (5 pages). Ordering information is given on any current masthead page.

Notes

Ring-Opening Reactions of Aziridines with Organometallics

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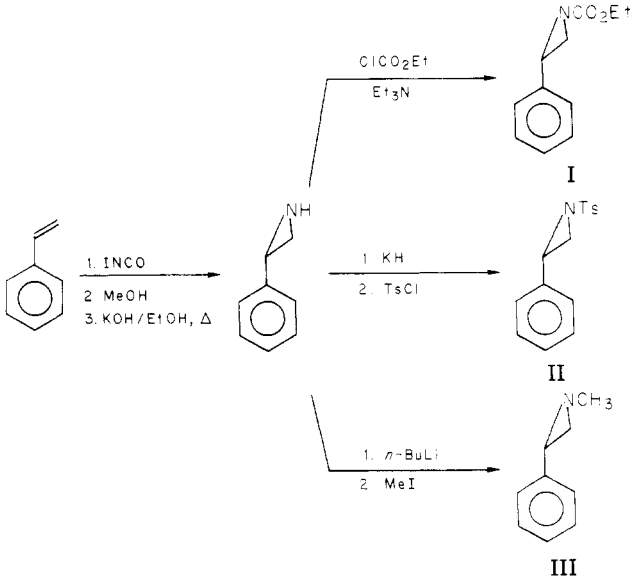
In our efforts to further the use of small-ring heterocycles in the synthesis of alkaloid systems, we initiated studies to ascertain the ability of various organometallics to effect cleavage reactions of N-substituted aziridines.[1] While the study was primarily aimed at defining the nature of the metal required to effect this transformation under the mildest of conditions, we were also concerned with the regiochemical outcome of this reaction when a choice between attack at a secondary carbon bearing a phenyl group or attack at a primary carbon was possible.

It should be noted that, while the reactions of epoxides with organometallics have been relatively well studied,[2] very few literature guidelines exist for conducting similar reactions on the nitrogen counterparts of these small-ring heterocycles. The only prominent reactions in this context are (a) the ring opening of aziridines in polar solvents by stabilized carbanions such as those derived from malonic esters to afford substituted α-pyrrolidones, (b) a report of attack at the ring carbon of N-carbethoxyaziridine by trityllithium, and (c) the observation that lithium dibutylcuprate gives ring-carbon attack for a rather special N-acylaziridine whereas the corresponding magnesium and lithium reagents react at the carbonyl group.[3]

The N-substituted derivatives of 2-phenylaziridine were chosen as the substrates for our studies, since these compounds would clarify the extent to which bond rupture in the transition state is guided by resonance stabilization.

Scheme I



† Fellow of the Alfred P. Sloan Foundation.

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