Synthesis of 1-(2,2,2,-Trichloroethyl)-2-(fluoromethyl)-3,4,5,6-tetramethylbenzene (7). To a stirred solution of 320 mg (0.90 mmol) of **5** in 10 mL of acetonitrile was added 400 mg of anhydrous AgF. Stirring was continued for 1 h at room temperature, followed by filtration and evaporation of the solvent. The residue was extracted with pentane, leaving after removal of the pentane in vacuo 240 mg of a yellow oil which contained 0.52 mmol (yield 58%, determined by ^1H NMR spectroscopy using benzene as reference) of **7**. Further purification could not be achieved due to the instability of **7** at room temperature (when stored at −40 °C, no decomposition was observed after several days). Also attempts to crystallize **7** (pentane and methanol, −40 °C) were unsuccessful. The sample of **7** obtained was free of extraneous absorptions in the methylene region of the ^1H NMR spectrum. Spectroscopic data for **7**: ^1H NMR (CCl_4) δ 5.57 (d, J_{HF} = 49 Hz, 2 H), 4.28[12] (s, 2 H), 2.35–2.23 (overlapping signals, probably with J_{HF} couplings, 12 H) (also see Table I); ^{13}C NMR (acetone-d_6) δ 137.8 136.9, 135.8, 135.3, 132.6, 129.1, 101.4 (CCl_3), 80.7 (dt, J_{CF} = 160 Hz, J_{CH} = 150 Hz), 52.5 (t, J_{CH} = 132 Hz), 19.6 (q), 17.5 (q), 16.9 (q), 16.8 (q) (see also Table I); exact mass calcd for $C_{13}H_{16}{}^{35}Cl_3F$ (M^+) 296.030, found 296.031.

Acknowledgment. We are grateful to Dr. Per Martinson, Mölndal, for helpful comments on our results.

Registry No. **4**, 76036-49-2; **5**, 70130-76-6; **6**, 70130-74-4; **7**, 76036-50-5.

(12) In the 100-MHz ^1H NMR spectrum this absorption is split into a doublet (J_{HF} = 0.8 Hz).

Antileukemic C-15-Functionalized Ambrosanolides from *Rudbeckia mollis*[1]

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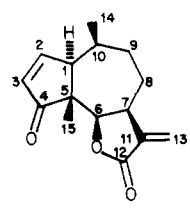
Four new C-15-functionalized ambrosanolides were isolated from *Rudbeckia mollis*. The structure and stereochemistry of the principal lactone constituent rudmollin (**2a**) were deduced by a combination of chemical and spectroscopic methods and by X-ray crystallography. Minor lactones 4-acetoxyrudmollin (**2b**), 15-acetoxyrudmollin (**2c**), and rudmollitrin (**4b**) were also obtained. Rudmollin and 15-acetoxyrudmollin exhibited activity in the P-388 lymphoid leukemia system.

Ambrosin (1, Chart I), damsin (2,3-dihydroambrosin), and some of their relatives[2] possess cytoxic or antitumor activity,[3,4] and much recent effort has been devoted to their synthesis.[5] Members of this group of sesquiterpene lactones, the ambrosanolides, are almost exclusively found in subtribe Ambrosiinae of Heliantheae[6] and appear to be responsible for the allergic contact dermatitis produced by various species of this subtribe.[4,7,8]

Chart I



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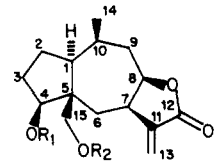
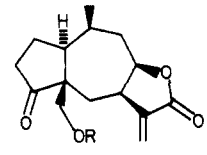
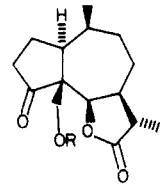


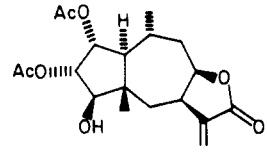
2a, R_1, R_2 = H
b, R_1 = Ac, R_2 = H
c, R_1 = H, R_2 = Ac
d, R_1, R_2 = Ac
e, R_1, R_2 =



3a, R = H
b, R = Ac



4a, R = H
b, R = Ac



5

In continuing our search for biologically active lactones[9] we had occasion to study *Rudbeckia mollis* Ell., a coneflower found in the coastal plain of Alabama, south Georgia, and north Florida. We now report isolation and

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

(2) For references to the chemistry, see: Fischer, N. H.; Oliver, E. J.; Fischer, H. D. *Fortschr. Chem. Org. Naturst.* **1979**, *38*, 47.

(3) Lee, K.-H.; Huang, E.-S.; Piantadosi, C.; Pagano, J. S.; Geissman, T. A.; *Cancer Res.* **1971**, *31*, 1649.

(4) Rodriguez, E.; Towers, G. H. N.; Mitchell, J. C. *Phytochemistry* **1976**, *15* 1573.

(5) Kretchmer, R. A.; Thompson, W. J. *J. Am. Chem. Soc.* **1976**, *98*, 3379. Marshall, J. A.; Ellison, R. A. *Ibid.* **1976**, *98*, 4312. de Clercq, P.; Vandewalle, M. *J. Org. Chem.* **1977**, *42*, 3447. Kok, P.; de Clercq, P.; Vandewalle, M. *Bull. Soc. Chim. Belg.* **1978**, *87*, 615. Demuynck, M.; de Clercq, P.; Vandewalle, M. *J. Org. Chem.* **1979**, *44*, 4863. Grieco, P.; Ohfune, Y.; Majetich, G. *J. Am. Chem. Soc.* **1977**, *99*, 7393. Lansbury, P. T.; Serelis, A. K. *Tetrahedron Lett.* **1978**, 1909. Semmelhack, M. F.; Yamashita, A.; Tomeschi, J. C.; Hirotsu, K. *J. Am. Chem. Soc.* **1978**, *100*, 5565. Wender, P. A.; Eisenstat, M. A.; Filosa, M. P. *Ibid.* **1979**, *101*, 2196. Quallich, G. J.; Schlessinger, R. H. *Ibid.* **1979**, *101*, 7627.

(6) Exceptions to the generalizations are as follows. (a) The discovery of an ambrosanolide among the plethora of sesquiterpene lactones in *Inula helenium* and *Pulicaria crispa*: Bohlmann, F.; Mahanta, P. K.; Jakupovic, J.; Rastogi, R. C.; Natu, A. A. *Phytochemistry* **1978**, *17*, 1165; Bohlmann, F.; Knoll, K.-H.; El-Emary, N. A. *Ibid* **1979**, *18*, 1231. (b) The isolation of an ambrosanolide stevin from *Stevia rhombifolia* (Eupatorieae): Rios, T.; Romo de Vivar, A.; Romo, J. *Tetrahedron* **1967**, *23*, 4265.

(7) E. Rodriguez, *Rev. Latinoam. Quim.* **1978**, *9*, 125.

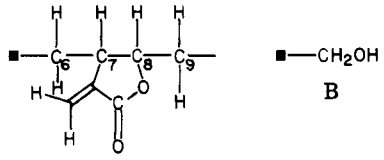
(8) Hausen, B. M. *Allergologie* **1979**, *2*, 143.

(9) Previous paper: Herz, W.; Govindan, S. V.; Blount, J. F. *J. Org. Chem.* **1981**, *46*, 761.

0022-3263/81/1946-1356$01.25/0

structure determination of a new family of C-15-oxygenated ambrosanolides from this species which belongs to subtribe Helianthinae rather than Ambrosiinae. Two of them have exhibited activity against P-388 lymphoid leukemia in vivo.

Rudmollin (**2a**, $C_{15}H_{22}O_4$), the major constituent of the plant, was an α-methylene α,β-unsaturated γ-lactone on the basis of the IR spectrum (bands at 1750 and 1650 cm^{-1}) and the typical narrowly split doublets appearing in the ^1H NMR spectrum at 6.29 and 5.67 ppm (H-13). By irradiation at these frequencies, H-7 of partial structure A

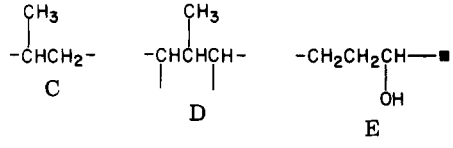


A

B

(numbering as in final formula) could be located at 3.18 ppm; this signal was split so as to indicate the presence of three vicinal protons. Two of these, H-6a and H-6b at 2.10 and 1.98 ppm, constituted the AB part of an ABX system (J_{AB} = 13 Hz) and hence represented the protons of a methylene group next to a quaternary center; the third, H-8 at 4.69 ppm and hence the proton under the lactone oxygen, was additionally coupled to two protons (H-9a and H-9b), giving rise to partially obscured multiplets. The carbon signal corresponding to H-8 was identified by single-frequency resonance decoupling (SFRD) as a doublet at 80.09 ppm and C-6 as a triplet at 36.09 ppm.

Rudmollin was a diol (strong IR absorption at 3420 cm^{-1}) as shown by preparation of a diacetate, **2d**. Acetylation was accompanied by downfield shifts of a one-proton triplet at 4.09 ppm associated with a secondary hydroxyl group (carbon doublet at 79.36 ppm by SFRD) and a two-proton AB quartet of a CH_2OH group (carbon triplet at 64.61 ppm) attached to quaternary carbon as in B because of the absence of further splitting. These conclusions were corroborated by spectroscopic examination (Tables I and II) of the two isomeric monoacetates **2b** and **2c** which were minor constituents of the plant extract. Each of these was converted to the same diacetate, **2d**. The remaining obvious feature in the ^1H NMR spectra of **2a–d** was a methyl doublet near 1 ppm; in pyridine-d_5 solution the signal of the proton responsible for the splitting of the methyl signal of rudmollin was clearly identifiable as a multiplet at 1.80 ppm arising from coupling to two additional protons (J = 12, 5 Hz) as in partial structure C or D.[10]



C D E

Oxidation of rudmollin with Jones reagent at 0 °C furnished two products. One of these was an acetonide **2e** derived from starting material; the second (**3a**) was a cyclopentanone (new IR band at 1740 cm^{-1}). Presence of a cyclopentanol moiety in the parent compound was also demonstrated by oxidation of **2c** to **3b** in whose ^{13}C NMR spectrum a carbonyl singlet at 214.80 ppm had replaced the doublet of **2c** at 77.55 ppm. Additional changes in the carbon spectrum accompanying this conversion were the downfield shifts of a triplet (from ~29 to ~36 ppm) and a singlet (from ~47 to ~54 ppm). Hence, these signals represented the two carbons α to the new ketone group. Moreover, in the ^1H NMR spectra of **3a** and **3b**, one of the protons associated with the new triplet near 36 ppm was responsible for a multiplet near 2.6 ppm whose appearance revealed that it was spin-coupled to one gem and two vicinal protons. Consequently, partial structure E which is included in a five-membered ring was also present in rudmollin.

Now the ^{13}C NMR spectra of **2a–d** contained only one quartet, two doublets, and one singlet in addition to the signals required by A, B, and E. Hence, partial structure D was ruled out, and the one quarternary carbon atom represented by the singlet at 48.57 ppm had to do triple duty in partial structures A, B, and E. Combination of these three to satisfy this requirement and insertion of C together with the one CH group found in the ^{13}C NMR spectrum but so far unaccounted for in the partial formulas led unequivocally to the pseudoguaianolide formula **2a** for rudmollin and to **2b** and **2c** for the two naturally occurring monoacetates.

As regards the stereochemistry of **2a** and its congeners, the couplings involving H-6, H-7, and H-8 and the values of $J_{7,13}$ definitely indicated that the γ-lactone ring was cis fused; for example, they essentially parallel those found in hymenograndin (**5**).[11] Facile formation of an acetonide from **2a** required that the secondary hydroxyl and the hydroxymethyl groups be cis; moreover, because acetylation and oxidation resulted in significant chemical shifts of one H-6 signal, namely, that exhibiting the smaller coupling to H-7, we deduced (model) that the hydroxyl and the hydroxymethyl groups were also cis to the lactone ring.

As the NMR signals necessary for investigating the stereochemistry at C-10 and C-1 were generally obscured, proof of the stereochemistry at these two centers presented problems. However, all naturally occurring pseudoguaianolides—whether helenanolides (with the C-10 methyl group α) or ambrosanolides (with the C-10 methyl group β)—are trans-fused bicyclo[5.3.0]decanes with H-1 α, and the positive Cotton effect of **3b** near 293 nm due to the n,π^* transition of the ketone indicated that rudmollin and its derivatives were no exception to this generalization.[12] With regard to the stereochemistry at C-10, it has already been mentioned that in C_6D_5N, H-10 of **2a** was seen at 1.80 ppm with coupling constants to its neighbors of 12, 5, and ~0 Hz. From the models, this seemed to accord somewhat more satisfactorily with H-1α and H-10β than with H-1α and H-10α (cf. $J_{1,10}$ = 12, $J_{9,10}$ = 4.5 and >1 Hz in hymenograndin[11]); hence, rudmollin and its congeners were initially thought to be helenanolides rather than ambrosanolides.[13]

To place the surprising discovery of pseudoguaianolides in a *Rudbeckia* species on a secure footing and to establish their stereochemistry at C-10, we undertook an X-ray

(10) Apparently one of the coupling constants between H-10, on the one hand, and H-1, H-9a, or H-9b (none of which was directly observable in the ^1H NMR spectrum) was vanishingly small (vide infra).

(11) Herz, W.; Govindan, S. V.; Bierner, M. W.; Blount, J. F. *J. Org. Chem.* **1980**, *45*, 493.

(12) 5,10-Trans-fused helenanolides and ambrosanolides like tetrahydrohelenalin or tetrahydroambrosin exhibit positive Cotton effects as do *trans*-hexahydroindan-1-ones of the same absolute configuration and 17-keto steroids.

(13) An attempt to verify the orientation of the C-10 methyl group by studying the lanthanide-induced shifts of **3a** (0.4 M solution of Eu(fod)$_3$) gave ambiguous results (see Table I, footnote *c*), as the H-10 and the H-14 signals both experienced a small paramagnetic shift, the former somewhat greater (0.4 ppm) than the latter. However, considerably larger paramagnetic shifts were observed for H-6a, H-6b, and H-7, possibly because the hydroxyl group on C-15 is directed away from the remainder of the molecule in solution just as it is in the crystal (see Figure 1).

Table I. ^{1}H NMR Spectra[a]

atom	compound									
	2a	2a[b]	2b	2c	2d	2e	3a[c]	3b	4a[d]	4b
H-2	1.5 m[f,g]	*e*	2.30 m[f,g]	*e*	*e*	*e*	*e*	*e*	2.18 m[f,g]	*e*
H-3a	2.2 m[g]	2.0 m[g]	*e*	2.21 m[g]	2.23 m[g]	1.90	2.58 ddd	2.63 ddd	2.58 ddd	2.53 ddd[g]
H-3b	1.80 m[h]	1.90 m[h]	*e*	1.84 m	1.60 m[h]	1.90	*e*	2.25 m[g]	2.30 m[h]	*e*
H-4	4.05 t (9)	4.21 t	5.01 dd (9, 7.5)	3.92 q (9)[l]	5.02 dd (9.2, 8.2)	3.97 t (4.5)				
H-6a	2.13 dd[g] (13, 3.5)	2.24 m	1.72 dd	1.84 dd (15, 5)	1.82 m[i]	2.05 dd (15, 5)	2.34 dd[g] (15, 3)	2.57 dd (15, 4.5)	4.54 d (9)	4.42 d (8.5)
H-6b	1.98 t (18)[i]	2.24 m	1.94 m[g]	1.77 m[h]	1.65 m[h]	1.73 t (15)	1.73 t (11.5)	1.74 t (15)[h]		
H-7	3.18 m	3.20 dddd (2.5, 2, 7.5, 13)	3.21 m	3.18 m	3.2 m	3.25 m	3.05 m	3.03 m	2.38 m[h]	2.47 m[g]
H-8	4.69 ddd (11.5, 7.5, 4)	4.69 ddd	4.70 ddd	4.69 ddd	4.69 ddd (11, 7.5, 4)	4.76 ddd (12, 7.5, 4)	4.69 ddd	4.68 ddd	*e*	*e*
H-9a	2.13 m[g]	2.0 m[g]	2.19 m[g]	2.18 m[g]	2.19 m	2.15 ddd	2.22 m[g]	2.22 m[g]	*e*	*e*
H-9b	1.83 m[h]	1.89 m[h]	*e*	*e*	1.89 m[i]	1.57 m[e]	1.85 m[e]	1.74 m[h]	*e*	*e*
H-10	1.98 m[i]	1.80 ddq (12, 5, 7)	*e*	2.08 m	2.03 m[g]	1.84 m[e]	2.09 m	*e*	2.15 m[g]	2.20 m[g]
H-13a	6.29 d (2.5)	6.24 d	6.25 d	6.28 d	6.28 d	6.31 d	6.27 d	6.28 d		
H-13b	5.67 d (2)	5.29 d	5.60 d	5.68 d	5.62 d	5.70 d	5.68 d	5.65 d	1.31 d (6.5)[j]	1.25 d (6)[j]
H-14[j]	0.96 d (7)	0.97 d	1.04 d	1.00 d	1.00 d	0.94 d	1.11 d	1.10 d	1.10 d	1.05 d (7.5)
H-15	4.09 d (12), 3.69 d br (12, 2)[k]	4.23 d 3.91 d br	3.91 d 3.69 d	4.18 (2 p)	4.14[m]	3.92 d, 3.14 d	3.74[m]	4.44 d, 3.97 d	4.10 d, 3.91 d	4.49 d (11) 4.33 d (11)
misc			2.13 (Ac)[j]	2.10 (Ac),[j] 2.53 d (OH)	2.09, 2.00 (Ac)[j]	1.38,[j] 1.40[j]		2.03 (Ac)[j]	2.40 m (H-11)[h]	2.02 (Ac),[j] 2.37 m (H-11)[g]

[a] Run at 270 MHz in $CDCl_3$ unless specified otherwise with Me_4Si as internal standard. Shifts are in parts per million. Figures in parentheses are coupling constants in hertz. [b] In pyridine-d_5. [c] With 0.4 M Eu(fod)$_3$, H-6a at 3.69 dd, H-6b at 2.89 t, H-7 at 3.65 m, H-8 at 5.03 ddd, H-10 at 2.48 m, H-13 at 6.55 d and 5.90 d, H-14 at 1.32 d, H-14 at 1.32 d, H-15 at 5.06 (center of AB), H-3a at 3.15 dd, br, H-3b at ~2.8 m. [d] With 0.4 M Eu(fod)$_3$, H-3a at 3.09 ddd, H-6 at 5.63 d, H-7 at 2.92 m, H-8a at 2.14 m, H-8b at 1.98 m, H-10 at 2.39, H-11 at 3.40 dq (8.5, 7.5), H-13 at 1.63 d, H-14 at 1.17 d, H-15 at 5.63 d and 5.22 d. [e] Obscured or unidentified signal. [f] One of two protons. [g–i] Overlapping signals. [j] Intensity three protons. [k] Collapses to d on addition of D_2O. [l] Collapses to t on addition of D_2O. [m] Center of AB quartet.

Table II. ^{13}C NMR Spectra[a]

carbon	compound 2a	2b	2c	2d	3d	4b
1	44.19 d	44.37 d	44.59 d	44.59 d	46.31 d	47.55 d
2	23.13 t	23.47 t	22.98 t	23.34 t	21.45 t	24.46
3	30.16 t	27.13 t	29.42 t	26.77 t	36.64 t[c]	37.38 t
4	79.36 d[b]	79.69 d[c]	77.55 d[b]	77.83 d[b]	214.80	215.69
5	48.57	48.87	47.29	47.45	53.90	56.67
6	36.09 t[b]	36.01 t	36.04 t	35.93 t	36.23 t[c]	81.65 d
7	37.76 d[b]	37.42 d	37.21 d	37.16 d	37.75 d	46.18 d
8	80.09 d[b]	79.87 d[c]	79.90 d[b]	79.32 d[b]	79.04 d	24.77 t
9	36.51 t	34.64 t	34.12 t	34.16 t	34.37 t	32.05 t
10	30.51 d	30.20 d	30.18 d	30.05 d	30.44 d	33.94 d
11	141.67	140.26	140.15	139.66	139.47	40.05 d
12	169.50	169.38	169.66	169.12	168.88	178.34
13	121.42 t	122.50 t	122.80 t	122.76 t	123.04 t	14.69 q[b]
14	16.57 q	16.44 q	15.81 q	15.96 q	17.29 q	15.56 q[b]
15	64.61 t	63.57 t	64.29 t	63.77 t	65.13 t	63.60 t
Ac		170.50, 21.05 q	170.98, 21.08 q	170.75, 170.29, 20.96 q, 20.96 q	170.24, 20.55 q	169.78, 20.67 q

[a] Run at 67.9 MHz in $CDCl_3$ with Me_4Si as internal standard. Unmarked signals are singlets. Shifts are in parts per million. [b] Signal identified by single-frequency resonance decoupling. [c] Assignments interchangeable.

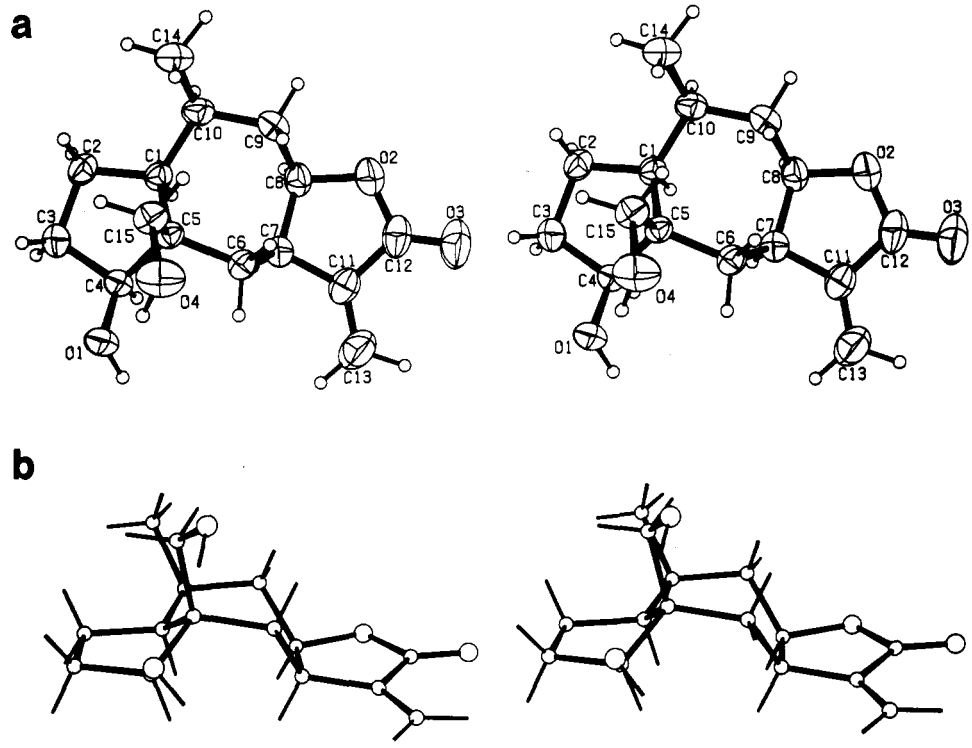


Figure 1. (a) Stereoscopic view of rudmollin (**2a**) with ellipsoids of thermal motion. (b) Side view of molecular framework.

analysis of rudmollin. Crystal data are listed in the Experimental Section. Figure 1a is a stereoscopic view of the molecule which also represents the absolute configuration because of the positive ketone Cotton effect of **3b** near 293 nm already referred to earlier. The C-10 methyl group is β; hence, rudmollin and its congeners are ambrosanolides rather than helenanolides. From Figure 1b it is seen that the large splitting involving H-10 apparently represents $J_{9\beta,10}$ rather than $J_{1\alpha,10}$, with the latter accounting for the splitting of 5 Hz and $J_{9\alpha,10}$ for the splitting of <1 Hz.

Tables IV–VIII listing final atomic and final anisotropic thermal parameters, bond lengths, bond angles, and selected torsion angles of rudmollin are available as supplementary material. The cycloheptane ring is a boat, with a central plane encompassing C(5)–C(6)–C(9)–C(10) and outer planes C(6)–C(7)–C(8)–C(9) and C(5)–C(1)–C(10).

Table III. Lactone Ring Torsion Angles (Degrees) of 2a

C(8)-O(2)-C(12)-C(11)	ω_1	−4.8
C(13)-C(11)-C(12)-O(3)	ω_2	1.4
C(11)-C(7)-C(8)-O(2)	ω_3	−8.7
C(6)-C(7)-C(8)-C(9)	ω_4	−3.3

The cyclopentane ring is an envelope with C(5) as the flap. This is shown more clearly in Figure 1b which presents a side view of the molecule. The γ-lactone ring is essentially flat, the sum of the internal torsion angles (Table VII) being only 27°. The sign of the very small C=C—C=O torsion angle (ω_2 of Table III) is positive. It is not paired with the sign of ω_3[14] and does not correspond to the sign of the (negative) Cotton effect associated with the n,π^*

(14) McPhail, A. T.; Sim, G. A. *Tetrahedron* **1973**, *29*, 1751.

transition of the α,β-unsaturated lactone. The situation is thus entirely analogous to that encountered in the case of hymenograndin which despite the different stereochemistry at C-10 and the extra substituents in the five-membered ring possesses the same conformation in the solid state and very similar torsion angles.[11] While the chirality of the lactone chromophore (ω_2) does not determine the sign of the Cotton effect, in violation of a generalization proposed by Beecham,[15] the sign of the Cotton effect does correlate with the sign of ω_3, in accordance with a suggestion made more recently.[16]

It now remains to consider the structure of a fourth lactone constituent of *R. mollis*, which was isomeric with **2b** and **2c** and which we have called rudmollitrin (**4b**). The presence of an acetate ester function and a cyclopentanone was suggested by the mass spectrum (loss of 42 mass units), the double-strength IR band at 1735 cm^{-1}, and the ^1H and ^{13}C NMR spectra which exhibited the usual signals characteristic of an acetate methyl and a carbonyl singlet at 215.69 ppm (Tables I and II). This was supported by hydrolysis of rudmollitrin to **4a** ($C_{15}H_{24}O_4$) whose IR spectrum had an band at 1742 cm^{-1} in addition to a new hydroxyl stretching frequency. The γ-lactone ring of **4b** (IR band at 1770 cm^{-1}) was saturated because the narrowly split, low-field doublets in the ^1H NMR spectra of **2a–d** were absent and were replaced by a methyl doublet. In accordance with this, the ^{13}C NMR spectrum lacked the low-field olefinic signals of **2a–d** but had gained a new doublet and a new quartet. Concomitantly, the C-7 doublet and the lactone carbonyl singlet had experienced the expected paramagnetic shifts to 46.1, and 178 ppm, respectively (Table II).

The AB system centered at 4.41 ppm, its upfield shift on hydrolysis, and the carbon triplet at 63.60 ppm showed that the acetate function of **4b** was again attached to C-15; the appearance of the H-3 signal (clearly visible in the presence of shift reagent) and comparison of the ^{13}C NMR spectra of **3b** and **4b** also indicated that the cyclopentanone carbonyl was located on C-4. However, the multiplicity of the signal at 4.42 ppm dictated lactone ring closure to C-6 rather than C-8; this also accounted for the downfield shift of the C-5 and the upfield shift of the C-9 signals.

Because of the absence of unsaturation, the CD curve of rudmollitrin displayed a single Cotton effect due to the n,π* transition of the cyclopentanone. Its positive sign, comparable to that of **3b**, required trans fusion of the bicyclo[5.3.0] system if the logical assumption is made that the absolute configurations of **2a–d** and **4b** are the same. In that case the magnitude of $J_{6,7}$ (9 Hz) required cis fusion of the γ-lactone ring (cf. $J_{6,7}$ = 9 Hz for damsin and its analogues). As helenanolides and ambrosanolides do not co-occur naturally, the C-10 methyl group of **4b** was presumed to be β; this was reinforced by the coincidence of the ^{13}C NMR spectra of **4b** and damsin[17] if allowance is made for the acetoxylation of C-15 and the saturation of the 11,13-double bond in **4b**. Use of lanthanide shift reagent had the effect of rendering visible the H-11 signal of **4a**; the value of $J_{7,11}$ (8.5 Hz) suggested that the C-11 methyl group was probably α. Hence, rudmollitrin is 11,13-dihydroincanin (11,13-dihydroligulatin B).[19]

In tests carried out under the auspices of the National Cancer Institute, rudmollin and 15-acetoxyrudmollin displayed activity in vivo against P-388 lymphoid leukemia (max T/C 128 at 100 mg/kg for **2a** and 125 at 130 mg/kg for **2c**.

Experimental Section

Isolation of Constituents of *Rudbeckia mollis*. Aboveground material of *Rudbeckia mollis* Pursh (5.5 kg), collected by Dr. R. K. Godfrey on July 12, 1959, near Steinhatchee, Dixie Co., FL (Godfrey No. 58786 on deposit in the herbarium of Florida State University), was extracted with $CHCl_3$ and worked up in the usual fashion.[23] The viscous gum remaining after removal of the solvent (13 g) was adsorbed on 20 g of silicic acid (Mallinckrodt, 100 mesh) and chromatographed over 300 g of the same adsorbent packed in $CHCl_3$–toluene (1:1), 200-mL fractions being collected as follows: fractions 1–5, $CHCl_3$-toluene (1:1); 6–11, $CHCl_3$; 12–17, $CHCl_3$–MeOH (99:1); 18–23, $CHCl_3$–MeOH (97:3); 24–30, $CHCl_3$–MeOH (19:1); 31–33 $CHCl_3$–MeOH (9:1).

Fractions 6–11 showed the presence of one major component. Further purification by preparative TLC and recrystallization from $CHCl_3$–hexane gave 50 mg of **2b**: mp 128–129 °C; $[\alpha]_D$ +80° (*c* 0.02, $CHCl_3$); IR (KBr) 3440, 1765, 1738, 1655, 1250 cm^{-1}. ^1H and ^{13}C NMR spectra are listed in Tables I and II. The low-resolution mass spectrum exhibited a relatively strong $M^+ + 1$ peak at *m/e* 309 instead of the molecular ion at *m/e* 308, but peaks diagnostic of formula $C_{17}H_{24}O_5$ were observed at *m/e* 290 ($M^+ - H_2O$), 266 ($M^+ - C_2H_2O$), 248 ($M^+ - C_2H_4O_2$), 230 ($M^+ - H_2O - C_2H_4O_2$), and 217.

Anal. Calcd for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84. Found: C, 66.15; H, 7.93.

Acetylation of 15 mg of **2b** (acetic anhydride–pyridine) and recrystallization from $CHCl_3$–hexane furnished 10 mg of **2d**: mp 120 °C; $[\alpha]_D$ +51.3° (*c* 0.053, $CHCl_3$); IR (KBr) 1750, 1735 (v s), 1660, 1240. ^1H and ^{13}C NMR spectra are listed in Tables I and II. The low-resolution mass spectrum exhibited diagnostic peaks at *m/e* 350 (M^+), 308 ($M^+ - C_2H_2O$), 290 ($M^+ - C_2H_4O_2$), 248 ($M^+ - C_2H_2O - C_2H_4O_2$), 230 ($M^+ - 2\ C_2H_4O_2$), 188, 93, and 79.

Fractions 12–17 exhibited one major spot on TLC. The substance (**4b**) was recrystallized from $CHCl_3$–hexane: yield 0.10 g; mp 140–141 °C; $[\alpha]_D$ +10.8° (*c* 0.17, $CHCl_3$); CD $[\theta]_{302}$ +3080 (max); IR (KBr) 1770, 1735 (double strength), 1240 cm^{-1}. ^1H and ^{13}C NMR spectra are listed in Tables I and II.

Anal. Calcd for $C_{17}H_{24}O_5$: mol wt 308.1623. Found: mol wt (mass spectrum) 308.1609 (2.7%).

Other significant peaks in the high-resolution mass spectrum were at *m/e* (composition, relative intensity) 266 ($C_{15}H_{22}O_4$, 21.1), 265 ($C_{15}H_{21}O_4$, 34.8), 248 ($C_{15}H_{20}O_3$, 67.5), 247 ($C_{15}H_{19}O_3$, 7.2), 237 ($C_{14}H_{21}O_3$, 4.6), 235 ($C_{15}H_{19}O_3$, 10.1), 233 ($C_{14}H_{17}O_3$, 8.3), 230 ($C_{15}H_{18}O_2$, 5.8), 220 ($C_{14}H_{20}O_2$, 31.6), 219 ($C_{14}H_{19}O_2$, 19.4), 209 ($C_{11}H_{13}O_4$, 8.1), 206 ($C_{13}H_{18}O_2$, 25.8), 201 ($C_{14}H_{17}O$, 6.7), 193 ($C_{12}H_{17}O_2$, 56.5), 192 ($C_{13}H_{20}O$, 2.6), 192 ($C_8H_{16}O_5$, 18.9), 191 ($C_{10}H_{15}O_2$, 19.3), 179 ($C_{11}H_{15}O_2$, 10), 175 ($C_{12}H_{15}O$, 17), 173 ($C_{12}H_{13}O$, 4.8), 163 ($C_{10}H_{11}O_2$, 13.1), 153 ($C_9H_{13}O_2$, 17.2), 152 ($C_9H_{12}O_2$, 16.3), 151 ($C_9H_{11}O_2$, 11.6), 141 ($C_7H_9O_3$, 39.8), 133 ($C_{1/}H_{13}$, 29.7), and 125 ($C_8H_{13}O$, 100).

TLC of fractions 18–23 indicated the presence of one major component which was purified by preparative TLC and recrystallized from $CHCl_3$–hexane: yield of **2c** 0.10 g; mp 150 °C; $[\alpha]$ +136° (c 0.5, $CHCl_3$); IR (KBr) 3420, 1750, 1730, 1652, 1250 cm^{-1}. The mass spectrum exhibited a relatively weak $M^+ + 1$ peak instead of the molecular ion at *m/e* 308. ^1H and ^{13}C NMR spectra are listed in Tables I and II.

(15) Beecham, A. F. *Tetrahedron* **1972**, *28*, 5543.
(16) Cox, P. J.; Sim, G. A. *J. Chem. Soc., Perkin Trans. 2* **1977**, 255.
(17) Herz, W.; Gage, D.; Kumar, N. *Phytochemistry*, in press. Differences between the values for **4b** in Table II and those for damsin are the upfield shifts of C-9 (1 ppm), C-3 (1 ppm), C-5 (1 ppm), C-7 (1.5 ppm), C-8 (1 ppm), and C-9 (1 ppm) and a downfield shift for C-4 (3 ppm) in addition to the expected larger shifts for C-11, C-12, C-13, and C-15.
(18) As in the case of **3a**, the effect of lanthanide shift reagent on **4a** was difficult to interpret, with H-15, H-11, H-6, and H-7 exhibiting decreasing paramagnetic shifts in this order (see Table I, footnote *d*). Shifts of H-10, H-13, and H-14 were small.
(19) Incanin (ligulatin B) has been isolated in small amounts from several *Parthenium* species.[20-22] Its dihydro derivative has not been described as catalytic hydrogenation results only in double bond isomerization to the 7,11-position.[21]
(20) Romo de Vivar, A.; Guerrero, C.; Wittgreen, G. *Rev. Latinoam. Quim.* **1970**, *1*, 39.
(21) Rodriguez, E.; Yoshioka, H.; Mabry, T. J. *Phytochemistry* **1971**, *10*, 1145; *Rev. Latinoam. Quim.* **1972**, *2*, 184.
(22) Rodriguez, E. *Biochem. Syst. Ecol.* **1977**, *5*, 207.
(23) Herz, W.; Högenauer, G. *J. Org. Chem.* **1962**, *27*, 905.

Anal. Calcd for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84. Found: C, 66.02; H, 7.00. Calcd for $C_{17}H_{25}O_5$: mol wt (+H) 309.1702. Found: mol wt (+H, mass spectrum) 309.1705.

Other significant peaks in the mass spectrum were at m/e (composition, relative intensity) 290 ($C_{17}H_{22}O_4$, 0.6), 248 ($C_{15}H_{20}O_3$, 3.5), 246 ($C_{15}H_{18}O_3$, 3.3), 233 ($C_{14}H_{17}O_3$, 5.1), 230 ($C_{15}H_{18}O_2$, 11.0), 218 ($C_{14}H_{18}O_2$, 5.8), 217 ($C_{14}H_{17}O_2$, 13.3), 215 ($C_{14}H_{15}O_2$, 10.3), 204 ($C_{10}H_{16}O_2$, 13.6), 189 ($C_{12}H_{13}O_2$, 14.7), 185 ($C_{14}H_{17}$, 13.7), 178 ($C_{11}H_{14}O_2$, 11.6), 171 ($C_{13}H_{15}$, 15.2), 150 ($C_{10}H_{14}O$, 27.1), 147 ($C_{11}H_{15}$, 20.6), 145 ($C_{11}H_{15}$, 54.4), 144 ($C_{11}H_{12}$, 21.2), 143 ($C_{11}H_{11}$, 29.2), 137 ($C_9H_{13}O$, 15.6), and 135 ($C_9H_{11}O$, 15.2).

Acetylation of 20 mg of **2c** gave 17 mg of **2d**, mp 120 °C.

Fractions 24–30 contained **2a**, the major constituent of the extract. Recrystallization from $CHCl_3$–hexane afforded colorless cubes: yield 8.0 g; mp 210–211 °C; $[\alpha]_D$ +110.4° (*c* 0.5, pyridine); CD (MeOH) $[\Theta]_{250}$ −5850 (min), $[\Theta]_{212}$ +240 000 (last reading); IR (KBr) 3390, 1750, 1655, and 825 cm^{-1}. ^1H and ^{13}C NMR spectra are listed in Tables I and II. The mass spectrum exhibited the $M^+ + 1$ rather than the M^+ ion.

Anal. Calcd for $C_{15}H_{22}O_4$: 67.65; H, 8.33. Found: C, 67.40; H, 8.49. Calcd for $C_{15}H_{23}O_4$: mol wt (+H) 267.1595. Found: mol wt (+H, mass spectrum) 267.1589 (3.3%).

Other significant peaks in the high-resolution mass spectrum were at m/e (composition, relative intensity) 248 ($C_{15}H_{20}O_3$, 4.7), 234 ($C_{14}H_{18}O_3$, 6.1), 233 ($C_{14}H_{17}O_3$, 28.9), 231 ($C_{15}H_{19}O_2$, 9.3), 230 ($C_{15}H_{18}O_2$, 35.4), 218 ($C_{14}H_{18}O_2$, 20.7), 217 ($C_{14}H_{17}O_2$, 14.2), 216 ($C_{14}H_{16}O_2$, 14.3), 215 ($C_{14}H_{15}O_2$, 68.5), 212 ($C_{15}H_{16}O$, 13.6), 204 ($C_{13}H_{16}O_2$, 15.7), 197 ($C_{14}H_{13}O$, 24.6), 191 ($C_{12}H_{15}O_2$, 42.5), 189 ($C_{1H13}O_2$, 100), 188($C_{12}H_{12}O_2$, 79.9), 187 ($C_{13}H_{15}O$, 43), 178 ($C_{11}H_{14}O_2$, 68.7), 176 ($C_{12}H_{16}O$, 30.2), 175 ($C_{11}H_{11}O_2$, 30.1), and 174 ($C_{12}H_{14}O$, 35.9).

Acetylation of 150 mg of **2a** gave 48 mg of **2d**, mp 120 °C.

Oxidation of 2a. A solution of 50 mg of **2a** in 2 mL of acetone was oxidized with Jones reagent at room temperature, the reaction being monitored by TLC. The usual workup gave a gum containing two products (TLC) which were separated by preparative TLC. The less polar fraction was a gum (**2e**) which could not be induced to crystallize: yield 10 mg; IR ($CHCl_3$) 1760, 1660, 1280, 1245, 1170, 1130, 1062 cm^{-1}. The ^1H NMR spectrum is listed in Table I. The low-resolution mass spectrum exhibited the molecular ion at m/e 306 and other peaks characteristic of an acetonide at m/e 291 ($M^+ - CH_3$), 277 ($M^+ - CHO$), 240 ($M^+ - C_3H_6O$), 230, 185, 105, and 91. The substance was identical with material prepared by allowing **2a** to stand in dry acetone with 2 drops of concentrated H_2SO_4.

The more polar substance, **3a**, was recrystallized from $CHCl_3$–hexane: yield 20 mg; mp 158–159 °C; IR ($CHCl_3$) 3500, 1760, 1740, 1600, 1280 cm^{-1}. The ^1H NMR spectum is listed in Table I. The low-resolution mass spectrum exhibited a weak molecular ion at m/e 264; other significant peaks occurred at m/e 246, 233, 215, 190, and 91.

Anal. Calcd for $C_{15}H_{20}O_4$: mol wt 264.1361. Found: mol wt (mass spectrum) 264.1353.

Oxidation of 2c. Reaction of 50 mg of **2c** with Jones reagent in dry acetone at room temperature, workup in the usual manner, and recrystallization from $CHCl_3$–hexane afforded **3b**: yield 45 mg; mp 160 °C; $[\alpha]_D$ +120° (*c* 0.04, $CHCl_3$); IR ($CHCl_3$) 1755, 1745, 1652 (sh), 1250 cm^{-1}; CD (MeOH) $[\Theta]_{293}$ +7340 (max), $[\Theta]_{247}$ −2450 (min), $[\Theta]_{214}$ +26 900 (last reading). ^1H and ^{13}C NMR spectra are listed in Tables I and II. The mass spectrum exhibited a weak $M^+ + 1$ peak at m/e 307 rather than the molecular ion at m/e 306, but stronger peaks diagnostic of the formula $C_{17}H_{22}O_5$ occurred at m/e 264 ($M^+ - C_2H_2O$) and 246 ($M^+ - C_2H_4O$).

Anal. Calcd for $C_{17}H_{23}O_5$: mol wt (+H) 307.1545. Found: mol wt (+H) 307.1532.

Hydrolysis of 4b. A solution of 20 mg of **4b** in a few drops of MeOH was stirred with 5 mg of KOH in 2 mL of H_2O at ambient temperature for 3 h, acidified with acetic acid, and extracted with ethyl acetate. The washed and dried extract was evaporated. The residue (**4a**) was purified by preparative TLC and recrystallized from $CHCl_3$–hexane: yield 10 mg; mp 178–179 °C; IR (KBr) 3410, 1742, 1210, 900 cm^{-1}; mass spectrum, m/e 266, 248 ($M^+ - H_2O$), 236 ($M^+ - CH_2O$), 218 ($M^+ - H_2O - CH_2O$), 191, 163, 119, 105, 91. The ^1H NMR spectrum is listed in Table I.

Anal. Calcd for $C_{15}H_{22}O_4$: mol wt 266.1517. Found: mol wt (mass spectrum) 266.1517.

X-ray Analysis of Rudmollin. Single crystals of rudmollin (**2a**), obtained by slow recrystallization from $CHCl_3$–hexane, were orthorhombic (space group $P2_12_12_1$) with a = 9.248 (2) Å, b = 10.828 (2) Å, c = 13.489 (2) Å, and d_{calcd} = 1.310 g cm^{-3} for Z = 4 ($C_{15}H_{22}O_4$, M_r = 266.34). The intensity data were measured on a Hilger-Watts diffractometer (Ni-filtered Cu Kα radiation, θ–2θ scans, pulse-height discrimination). A crystal measuring approximately 0.35 × 0.40 × 0.6 mm was used for data collection. A total of 1075 reflections were measured for $\theta < 57°$ of which 1057 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved by a multiple-solution procedure[24] and was refined by full-matrix, least-squares methods. Five reflections which were strongly affected by extinction were excluded from the final refinement and difference map. In the final refinement anisotropic thermal parameters were used for the nonhydrogen atoms, and isotropic temperature factors were used for 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 = 0.031 and R_w = 0.044 for the remaining 1052 observed reflections. The final difference map has no peaks greater than ±0.1 e A^{-3}.

Registry No. 2a, 76467-15-7; **2b**, 76467-16-8; **2c**, 76467-17-9; **2d**, 76467-18-0; **2e**, 76467-19-1; **3a**, 76467-20-4; **3b**, 76479-90-8; **4a**, 76479-77-1; **4b**, 76467-21-5.

Supplementary Material Available: Tables listing final atomic (Table IV) and anisotropic thermal (Table V) parameters, bond lengths (Table VI), bond angles (Table VII), and selected torsion angles (Table VIII) for **2a** (4 pages). Ordering information is given on any current masthead page.

(24) Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr., Sect. A* 1971, A27, 368.