repeatedly from ethanol to give 18 mg of pure artemetin: mp 162–163°; mol wt (by mass spectrometry) 388; nmr signals 7.22 (d) and 6.97 (d) (J = 9.2, H-6′ and H-5′ respectively), 7.70 (H-2′), 6.48 (H-8), 3.96, 3.94, 3.92, and 3.88 ppm (4 methoxyls); and gave the color reaction previously reported for artemetin. The mixture melting point with an authentic sample[22] was not depressed. The methyl ether had mp 156–157°, mixture melting point with an authentic sample[22] undepressed. All other fractions were gums showing several spots. Repetition of the extraction with plant material collected in July 1969 at the same location gave the same results.

(22) W. Herz, *J. Org. Chem.*, **26**, 3014 (1961).

Registry No.—**1a**, 24694-79-9; **1b**, 24694-80-2; **1c**, 24694-81-3; **1d**, 24728-11-8; **1e**, 24694-82-4; **2a**, 24728-12-9; **3a**, 24728-13-0; **3b**, 24806-56-2; **3c**, 24694-83-5; **3d**, 24694-84-6; **3e**, 24694-85-7; **3f**, 24694-86-8; **3g**, 24728-14-1; **4a**, 24694-87-9; **5a**, 24694-88-0; **6a**, 24694-89-1; **7a**, 24694-90-4; **8**, 24694-91-5; **9a**, 24694-92-6; artemetin, 479-90-3.

Sesquiterpene Lactones and Lactone Glycosides from *Hymenoxys* Species[1,2]

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Received February 17, 1970

A group of interesting sesquiterpene dilactones has been isolated from some *Hymenoxys* species. Floribundin (**3**) and vermeerin (**2**), the latter previously found in South African *Geigeria* species, were isolated from the southwestern U. S. stock poison *H. richardsonii* and the South American *H. anthemoides*. *H. anthemoides* also yielded anthemoidin and themoidin which are dihydro derivatives of **2** and **3**, respectively. *H. greenei* gave **3** and the dilactone greenein (**6**). Separate collections of *H. odorata* afforded either a lactone glucoside hymenoxynin (**14a**) or the pseudoguaianolide glucoside paucin (**13a**) and a lactone lactal hymenolide (**8a**) which could be correlated with hymenoxynin and floribundin. Structures, stereochemistry, and conformations for all compounds were derived by chemical methods and extensive application of nmr techniques.

As a consequence of the discovery of a group of biogenetically "abnormal" sesquiterpene lactones, the so-called pseudoguaianolides, in some *Helenium* species,[5] the genera *Helenium* and *Gaillardia*, the latter adjoining *Helenium* in the taxonomic scheme of *Compositae* (tribe *Helenieae*, subtribe *Heleniinae*), have received careful scrutiny.[6,7] In general, elaboration of pseudoguaianolides seems characteristic of these two genera, although some exceptions have been noted.[6,8]

While *Helenieae* is thought by many to be a rather artificial assemblage not deserving of tribal status,[11] certain natural subdivisions exist. For example, it is generally agreed that the genus *Hymenoxys* is closely allied to *Helenium* and *Gaillardia*. Accordingly, chemical examination of *Hymenoxys* species appeared to be of interest. Knowledge of their sesquiterpene lactone content could conceivably contribute to a better understanding of phylogenetic relationships within the group. Moreover several representatives such as *Hymenoxys odorata* DC. and *H. richardsonii* (Hook) Ckll. var. *floribunda* (pingue bitterweed) are well-known stock poisons of the American southwest;[12] it seemed possible that sesquiterpene lactones might be responsible for their activity. We have therefore embarked on a study of this genus. In the following we report the results of our initial study of four *Hymenoxys* species. Work on other species is continuing.[13]

Results

Table I lists species included in the present investigation and the crystalline sesquiterpene lactones isolated

(1) Supported in part by a grant from the U. S. Public Health Service (GM-05814).

(2) Previous paper on Sesquiterpene Lactones: W. Herz and S. V. Bhat, *J. Org. Chem.*, **35**, 2605 (1970).

(3) To whom correspondence should be addressed.

(4) On leave of absence at Florida State University, 1967–1968.

(5) W. Herz, W. A. Rohde, K. Rabindran, P. Jayaraman, and N. Viswanathan, *J. Amer. Chem. Soc.*, **84**, 3857 (1962); W. Herz, A. Romo de Vivar, J. Romo, and N. Viswanathan, *ibid.*, **85**, 19 (1963).

(6) For reviews of work through 1966, see W. Herz, "Pseudoguaianolides in *Compositae*, Recent Advances in Phytochemistry," T. J. Mabry, R. E. Alston, and V. C. Runeckles, Ed., Appleton-Century-Croft, New York, N. Y., 1968, p 220; J. Romo and A. Romo de Vivar, "The Pseudoguaianolides, Progress in the Chemistry of Natural Products," L. Zechmeister, Ed. Springer Verlag, Vienna, Vol. 25, 1967, p 90.

(7) For the most recent paper on *Helenium* species, see W. Herz, P. S. Subramaniam, and N. Dennis, *J. Org. Chem.*, **34**, 2915 (1969).

(8) To these exceptions must now be added pulchellins B, C, E, and F from Western races of *G. pulchella* Foug. Recent work[9] has shown these sesquiterpene lactones to be eudesmanolides rather than pseudoguaianolides as originally supposed.[10]

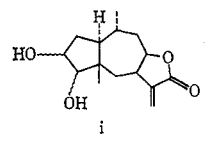
(9) H. Yoshioka, N. Dennis, W. Herz, and T. J. Mabry, *J. Org. Chem.*, **35**, 627 (1970).

(10) W. Herz and S. Inayama, *Tetrahedron*, **20**, 341 (1964); W. Herz and S. K. Roy, *Phytochemistry*, **6**, 661 (1969).

(11) A. Cronquist, *Amer. Midl. Natur.*, **53**, 478 (1955); O. Solbrig, *J. Arnold Arboretum*, **44**, 436 (1963).

(12) J. M. Kingsbury, "Poisonous Plants of the United States and Canada," Prentice-Hall, Englewood Cliffs, N. J., 1964.

(13) While our work was in progress, two other groups reported on constituents of certain *Hymenoxys* species. Thomas and Mabry[14] isolated a number of flavonoids from *H. scaposa* DC. whereas Romo and coworkers[15] obtained a new pseudoguaianolide odoratin (i) from a San Luis Potosi



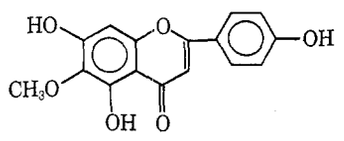
i

collection of *H. odorata*. Because we encountered different sesquiterpene lactones in collections of *H. odorata* from two separate localities (*vide infra*, Table I), the difference between the results reported by the Mexican workers and by us is not particularly surprising. Moreover, odoratin appears to be a possible precursor of the substances isolated by us (*vide infra*).

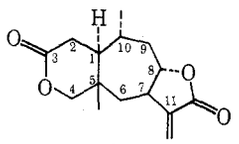
(14) M. B. Thomas and T. J. Mabry, *J. Org. Chem.*, **32**, 3254 (1967); *Tetrahedron*, 3675 (1968); *Phytochemistry*, **7**, 787 (1968).

(15) A. Ortega, A. Romo de Vivar, and J. Romo, *Can. J. Chem.*, **46**, 1538 (1968).

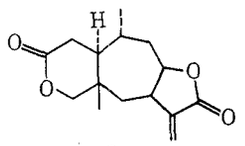
from them. In addition, the flavone hispidulin (**1**)[16] was found in *H. odorata* and *H. richardsonii*.



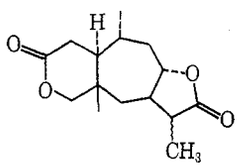
1



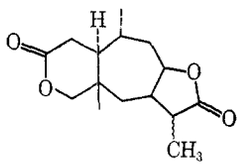
2



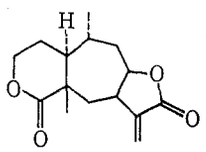
3



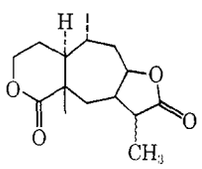
4a, methyl α
b, methyl β



5a, methyl β
b, methyl α



6



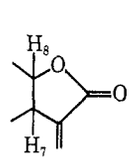
7

Vermeerin and Floribundin.—Two very similar compounds whose ir spectra indicated the presence of an α,β-unsaturated γ-lactone (1766, 1670 and 1757, 1656 cm^{-1}, respectively) and that of a δ-lactone (1729 and 1727 cm^{-1}, respectively) were isolated from *H. richardsonii*. Analysis of the nmr spectrum of the first substance (Table II) and other physical properties which will not be discussed in detail suggested that it might be identical with vermeerin, a sesquiterpene dilactone from *Geigeria aspera* Harv.[20] and *Geigeria africana* Gries[21] (*Compositae*, tribe *Inuleae*) to which formula **2** (devoid of stereochemistry) has been assigned. Direct comparison with an authentic sample of vermeerin established identity.[22]

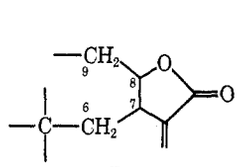
The gross structure of floribundin[22a] shown in formula **3**, stereoisomeric with that of vermeerin, was based on the detailed analysis of its nmr spectrum (Tables II and III) which immediately revealed the characteristic two doublets of the exocyclic methylene group at 6.28 (J = 2.5 Hz) and 5.60 ppm (J = 2.2 Hz). Frequency-swept decoupling experiments showed that the splitting was, as usual, caused by coupling to an allylic proton (complex multiplet at 3.21 ppm) which was in turn coupled vicinally to a proton of type –O–CH, the latter forming a symmetrical octet with degenerate central lines at 4.78 ppm (partial formula A).

Decoupling and tickling experiments showed that H-7 of A was coupled to two other protons which were responsible for two quartets in the high-field region, at 1.37 (J_1 = 15.5, J_2 = 3.5 Hz) and at 1.92 ppm (J_1 = 15.5, J_2 = 13.2 Hz). Because of the chemical shifts, these protons had to be attached to sp^3-type carbon atoms which do not carry any functional group, and because of the splitting constants, they had to be geminal.

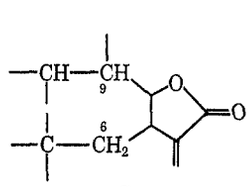
Unambiguous assignment of the environment of H-8 of A was difficult because the distribution of signals in the high field region was unsuitable. However, the shape of the H-8 multiplet indicated the presence of couplings (3.7 and 11.7 Hz) to two protons in the remaining part of the molecule. Now it could be assumed that the larger one of these interactions (11.7) was vicinal, but the magnitude of the smaller one (3.7 Hz) does not exclude the presence of an anomalous σ–σ interaction of the type ^4J. Hence A could be extended to B or C.



A



B



C

The nature of the δ-lactone ring was derived by considering other features of the nmr spectrum. The low-field region contained, in addition to the signals of H-8 and H-13, a typical AB system at 4.15 and 3.83 ppm (J_{AB} = 11.0 Hz) which could be ascribed to two protons of the –O–CH type. The occurrence of this system in the floribundin spectrum can be due only to a fragment –CO–OCH$_2$–C– which comprises part of the six-membered lactone ring. The low-field doublet exhibited a larger line width than the high-field doublet, indicating that a further long-range coupling was present. By means of tickling experiments it was established that the long-range coupling was due to the protons of a tertiary methyl group at 1.08 ppm. Hence partial formula D could be written for the δ-lactone ring.

The nmr spectrum of floribundin contains a separate one-proton quartet at 2.84 ppm (J_1 = 5.75, J_2 = 18.75 Hz, H-2a) coupled (tickling experiments) to two other protons. One of these (H-2b) formed a quartet at 2.14 (J_1 = 11.0, J_2 = 18.75 Hz), the second (H-1) a complex

(16) W. Herz and Y. Sumi, *J. Org. Chem.*, **29**, 3438 (1964). It has been shown recently[17] that the structure previously[18] attributed to dinatin is erroneous and that dinatin is identical with hispidulin. A synthesis of hispidulin (dinatin) has been recorded.[19]

(17) D. K. Bharadwaj, S. Neelakantan, and T. R. Seshadri, *Indian J. Chem.*, **4**, 173 (1966).

(18) S. Rangaswami and E. V. Rao, *Proc. Indian Acad. Sci., Sect. A*, **54**, 51 (1961).

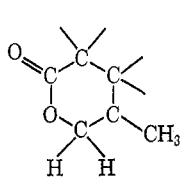
(19) P. S. Phadke, A. V. Rama Rao, and K. Venkataraman, *Indian J. Chem.*, **5**, 131 (1967).

(20) C. Rimington and G. C. S. Roets, *Onderstepoort J. Vet. Sci. Anim. Ind.*, **7**, 485 (1963).

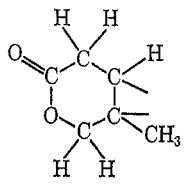
(21) L. A. P. Anderson, W. T. de Kock, K. G. R. Pachler, and C. V. D. Brink, *Tetrahedron*, **23**, 4153 (1967). We are grateful to Dr. de Kock for sending us an authentic sample.

(22) Vermeerin is the dilactone of the physiologically active vermeric acid whose occurrence in *Geigeria* species causes "vomiting disease" among sheep in South Africa.[21] The symptoms are apparently similar to those produced in livestock browsing on *H. richardsonii* in the U. S.[12] It is logical to assume that vermeerin and its congeners, or their precursors, are also responsible for the toxicity of this species.

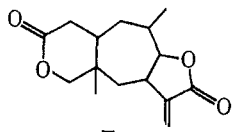
(22a) NOTE ADDED IN PROOF.—After submission of this manuscript, we learned that a compound with properties similar to those of floribundin had been isolated from *Psilostrophe cooperi* (Gray) Greene in the laboratory of Professor T. A. Geissman and assigned the same structure. This work has since been published, L. B. de Silva and T. A. Geissman, *Phytochem.*, in press. Direct comparison of floribundin and psilotropin in the laboratory of Professor T. J. Mabry established their identity.



D



E



F

multiplet hidden in the high-field region near about 1.66 ppm. The high absolute value of J_2 indicated that H-2a and H-2b were geminal protons on a sp^3-hybridized carbon bonded to a sp^2-hybridized carbon atom (σ–π interaction, enhancement of geminal coupling[23]). The latter must be the carbonyl group of the δ-lactone ring. Since J_1 of H-2b also arose through coupling of H-2b to H-1, partial formula D could be expanded to E.

The only other clearly visible signal in the spectrum of floribundin was that of a secondary methyl group. Combination of B or C with E then led to two alternative formulas **3** and F which differ in the location of the secondary methyl group. The former is clearly preferred on biogenetic grounds.

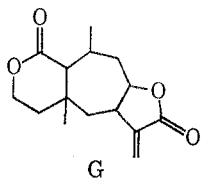
Derivation of partial formula C and therefore that of F, was based on the consideration that the smaller coupling exhibited by H-8 (3.7 Hz) might possibly be due to an anomalous long-range interaction. However, the existence of an σ–σ interaction in F with a value as high as 3.7 Hz would be quite improbable. Formula F could be definitely excluded on the basis of spin decoupling experiments which showed that H-8 did not interact with the methine proton of the fragment –CH–CH_3. Hence floribundin possesses structure **3** (exclusive of stereochemistry).

Anthemoidin and Themoidin.—Catalytic hydrogenation of vermeerin afforded a mixture of reduction products. The major product, dihydrovermeerin A (**4a**), mp 128°, $[\alpha]$ − 25.7°, was stable to base and was also obtained in quantitative yield by sodium amalgam–acetic acid reduction of vermeerin. A minor isomer, dihydrovermeerin B (**4b**), was identical with anthemoidin. Catalytic hydrogenation or sodium amalgam reduction of floribundin gave only one crystalline dihydro derivative (**5a**) which was identical with themoidin.

Greenein.—The infrared spectrum of greenein (bands at 1770, 1735, and 1660 cm^{-1}) also suggested the presence of partial structure A and a δ-lactone group. Detailed analysis of the nmr spectrum of greenein (Tables II and III) and frequency-swept decoupling experiments showed that the constitution of greenein differed from that of **2** and **3** in the location of the fragment –CO–O– of the six-membered lactone ring.

In addition to signals associated with B, the low-field region of the spectrum contained, at 4.13–4.62 ppm, signals of two protons of the type –O–CH– which had to be assigned to the fragment –CO–O–CH_2–. However, the appearance of this multiplet indicated that the protons of the methylene group in this fragment were coupled vicinally to at least two other protons. Hence two alternative formulae were possible, **6** (exclusive of stereochemistry) and G.

(23) L. M. Jackman and S. S. Sternhell, "Applications of Nuclear Magnetic Resonance in Organic Chemistry," 2nd ed, Pergamon Press, 1968.



G

When the spectra of floribundin and greenein are compared (Tables II and III), it becomes apparent that the chemical shifts of H-7 and H-8, as well as their vicinal and long-range couplings, are practically identical. This coincidence supports the conclusion that the configuration and conformation of ring B and the γ-lactone ring in floribundin and greenein are the same. On the other hand, H-6a and H-6b of greenein, which can be identified through their vicinal coupling constants with H-7, are considerably more deshielded, the former by 65 Hz. This deshielding effect relative to **3** can be explained in terms of formula **6**, but not in terms of G. Contrariwise, in a compound of formula G, one would expect a significant change in the chemical shifts of H-10 or of the C-10 methyl group, depending on the configuration at C-10, relative to that found in **3**, a situation which, as can be seen from Table II, does not prevail. Hence **6** is an appropriate expression for greenein.

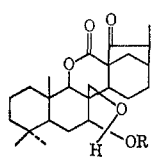
Hydrogenation of **6** afforded a dihydro derivative **7** different from **4a**, **4b**, **5a**, and **5b**. Paucity of material prevented further chemical work.

Hymenolide.—This substance **8a** had ir bands corresponding to the presence of a hydroxyl (3570, 3445 cm^{-1}) and an α,β-unsaturated γ-lactone group (1750, 1660 cm^{-1}); its nmr spectrum (see Experimental Section) exhibited signals characteristic of partial formula A, an ethoxyl group and two additional protons of type H–CO, one a singlet at 4.38 and one a doublet of doublets at 5.11 ppm.

Conversion of hymenolide to an acetate **8b**, a reaction which confirmed the presence of a hydroxyl group, was accompanied by a downfield shift of the doublet of doublets to 6.08 ppm. These unusual high paramagnetic shifts are characteristic of a hemiacetal hydrogen[24] which, because of the multiplicity of its signal, has to adjoin a methylene group.

Confirmation for the presence of such a hemiacetal linkage in hymenolide was provided by chromium trioxide–pyridine oxidation of **8a** to a dilactone **9** and by pyrolysis of **8b** to an anhydro derivative **10**. The nmr spectrum of the latter (Tables II and III) exhibited signals typical of the grouping $-CH_\gamma-CH_\beta=CH_\alpha-O-$, where H_α at 6.08, H_β at 4.70, H_γ at 2.07 ppm, $J_{\alpha,\beta}$ =

(24) Compare with the values of H-6 in the enmein derivatives iia (5.38), iib (4.72), and iic (6.15 ppm).[25]



iia, R = H
b, R = CH_3
c, R = Ac

(25) T. Kubota, T. Matsuura, T. Tsutsui, S. Uyeo, M. Takahashi, H. Irie, A. Numata, T. Okamoto, M. Natsume, Y. Kawazoe, K. Sudo, T. Ikeda, M. Tomoeda, S. Kanatomo, T. Kosuge, and K. Adachi, *Tetrahedron Lett.*, 1243 (1964).

TABLE I

SESQUITERPENE LACTONES FROM *Hymenoxys* SPECIES

Species	Compd	Molecular formula	Mp, °C	$[\alpha]_D$, deg	Species	Compd	Molecular formula	Mp, °C	$[\alpha]_D$, deg
H. richardsonii (Hook) Ckll. var. *floribunda* (Gray) Parker	Vermeerin[a]	$C_{15}H_{20}O_4$	145–146	−67.1	*H. greenei* (Ckll.) Rydb.	Floribundin			
	Floribundin	$C_{15}H_{20}O_4$	143	+84.0		Greenein	$C_{15}H_{20}O_4$	169–171	+114
H. anthemoides (Juss.) Cass.	Vermeerin[a]				*H. odorata* DC.	Hymenoxynin[b]	$C_{21}H_{34}O_9 \cdot H_2O$	125–128	−37.6
	Floribundin					Hymenolide[c]	$C_{17}H_{26}O_5$	136–138	−48.6
	Anthemoidin	$C_{15}H_{22}O_4$	220–221	−115		Paucin[c,d]	$C_{23}H_{32}O_{10}$	177–179	+64.4
	Themoidin	$C_{15}H_{22}O_4$	219–220	+61.8					

[a] Previously isolated.[20] [b] From Coahuila collection. [c] From N. M. collection. [d] Previously isolated.[28]

TABLE II

CHEMICAL SHIFTS IN NMR SPECTRA OF *Hymenoxys* LACTONES[a]

Compd	H-1	H-2a	H-2b	H-4a	H-4b	H-6a	H-6b	H-7	H-8	H-9a	H-9b	H-10	H-11	H-13a	H-13b	H-14	H-15
2	∼2.06	2.70	2.35	4.11	3.87	1.84	1.46	2.79	3.99	∼2.33	1.27–1.6	(∼1.8)		6.16	5.45	1.02	1.09
3	∼1.66 (1.63)	2.84	2.14	4.15	3.83	1.92	1.37	3.21	4.78	(∼2.14)	(1.90)	(1.75)		6.28	5.60	1.09	1.08
4a		2.67	2.33	4.05	3.82				3.95			∼1.70[b]	∼2.22[b]	1.17		0.98	1.05
4b	1.98	2.68	2.34	4.09	3.81				4.15			∼1.64[b]	∼2.62[b]	1.14		0.97	1.03
5a		2.84	2.15	3.95	3.76	1.51	∼1.15[c]	∼2.59[c]	4.73			∼1.69[b]	∼2.93[b]	1.11		1.05	1.11
5b		2.81		4.06	3.79				4.77				∼2.20[b]	1.32		1.06	1.07
6[d]						2.96	1.75	3.21	4.79			∼1.79[b]		6.28	5.70	1.15	1.27
10[e]	2.07	...[c]		4.38		1.74	1.51	3.52[f]	4.76	1.7–2.1[c]	1.7–2.1[c]	∼1.70		6.23	5.51	1.12	0.90

[a] First-order values from 100-MHz spectra (Varian HA-100), solvent $CDCl_3$, internal standard hexamethyldisiloxane (HMDS) except for **5b** and **10** (TMS). Chemical shifts given in δ (TMS) values using δ (HMDS) = 0.06 ppm. Values in parentheses are corresponding values from 220-MHz spectra (Varian HR-220). [b] From INDOR spectra. [c] Approximate value from double resonance experiment. [d] 2H-3, 4.13–4.63 ppm. [e] H-2, 4.70 ppm; H-3, 6.08 ppm; $-OCH_2CH_3$; $-CH_2-$ nonequivalent protons at 3.42–4.01 ppm, CH_3, triplet at 1.22 ppm (J = 7 Hz). [f] Hidden in the multiplet of $-CH_2-$ of ethoxyl.

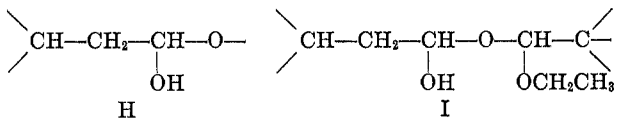
TABLE III

COUPLING CONSTANTS IN NMR SPECTRA OF *Hymenoxys* LACTONES[a]

Compd	$J_{1,2a}$[b]	$J_{1,2b}$[b]	$J_{1,10}$[c]	$J_{6a,7}$[d]	$J_{6b,7}$[d]	$J_{7,8}$	$J_{7,11}$[e]	$J_{7,13a}$	$J_{7,13b}$	$J_{8,9}$	$J_{8,9b}$	$J_{4,15}$[f]	$J_{1,15}$[f]	$J_{2a,b}$[b]	$J_{4a,b}$	$J_{6a,b}$[d]	$J_{13a,b}$[f]	$J_{11,13}$	$J_{10,14}$
2	5.6	11.5	(8.4)	2.75	11.0	9.5		3.4	3.1	4.0	11.5	0	0	17.6	11.0	15.0	0		6.1
	(5.4)	(12.6)		(2.5)	(11.4)	(10.5)				(4.0)	(10.5)			(18.3)		(15.4)			
3	5.75	11.0	(11.4)	13.25	3.5	8.0[g]		2.5	2.2	11.7[g]	3.7[g]	0	0	18.75	11.0	15.5	0		6.0
	(5.9)	(11.9)		(13.3)	(3.5)									(18.8)		(15.9)			
4a	5.8	12.0				9.2[h]	12.7			4.8[h]	10.9[h]	0		18.5	11.0			6.6	6.3
4b	5.5	12.5	7.3			10.5	7.5			5.0	10.5	0	0	17.85	11.0			7.5	6.7
5a	5.5	11.5		13.5	2.5	...[i]	8.5–9.0			...[i]	...[i]	0		18.6	11.0	15.2		7.5	6.1
5b	5.5					...[i]	...[j]			...[i]	...[i]	0		18.5	11.0			7.3	∼6.0
6				3.0	13.3	8.0[g,k]		2.65	2.30	11.75[g,k]	3.75[g,k]					15.3	0		6.0
10[l]			11.5	12.0	4.5	7.8[m]		2.4	2.1	...[m]	...[m]	0[n]	0			15.0	0		6.0

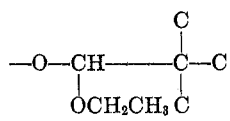
[a] First-order values from 100-MHz spectra (Varian HA-100), solvent $CDCl_3$. Values in parentheses correspond to splittings from 220-MHz spectra (Varian HR-220). Numbering of protons corresponds to that in Table II. All values taken directly from charts. [b] From multiplets of H-2a and H-2b. [c] From multiplets of H-1. [d] From multiplets of H-6a and H-6b. [e] From INDOR spectra. [f] From decoupling experiments; $J_{4,15} < 0.5$ Hz, $J_{13a,b}$ of **3** $\ll 0.5$ Hz. [g] Multiplets of H-8 identical at 100 and 200 MHz. [h] H-8 multiplet similar to that in **2**. [i] Multiplet of H-8 is virtual complex; $\Sigma \sim 23$ Hz in **5a**, ∼25 Hz in **5b**. [j] Could not be estimated from INDOR spectra. [k] From multiplet of H-8 which was practically identical with that of **3**. [l] $J_{1,2} = 1.6$ Hz; $J_{1,3} = 2.65$ Hz; $J_{3,4} = 0 \ll 0.5$ Hz; $J_{2,3} = 6.35$ Hz. [m] Multiplet of H-8 coincides with H-2 multiplet; splittings 7, 8, 10.5, 5.2 Hz. [n] $W_{1/2}$ (H-15) ∼ 0.8–0.9 Hz.

6.35, $J_{\alpha,\gamma}$ = 2.65, and $J_{\beta,\gamma}$ = 1.6 Hz (chemical shifts and coupling constants verified by spin decoupling).[26] Hence hymenolide contains partial formula H.



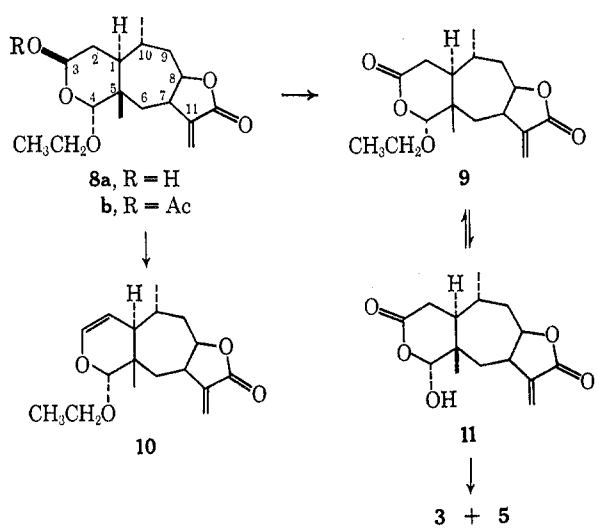
H I

Exposure of dehydrohymenolide **9** to dilute acid resulted in hydrolysis of the ethoxyl group; treatment of the product **11** with ethanolic HCl reconstituted **9**. This behavior, and the nmr spectra of **9** and **11** were consonant with the presence of the grouping



which, because of the presence of a lactone group in hymenolide and its empirical formula, must be combined with H into partial structure I. Hence, hymenolide must be **8a**, the nature of the seven-membered ring and its combination with I being established by spin-decoupling experiments on **10** (Tables II and III) in the manner described for floribundin.

Chemical evidence for the formulation of hymenolide as **8a** was provided as follows. Treatment of the lactol **11**, which is presumably in equilibrium with the aldehydo acid, with $NaBH_4$ in methanol resulted in formation of floribundin and themoidin. Hence, the configuration of hymenolide at C-1, C-5, C-7, C-8, and C-10 is the same as that of floribundin.



8a, R = H
b, R = Ac

9

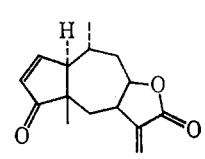
10

11

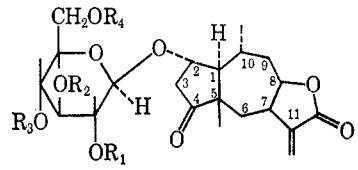
3 + 5

Paucin.—The empirical formula of a highly polar substance from the N. M. collection of *H. odorata*, its ir spectrum (strong hydroxyl absorption, α,β-unsaturated lactone at 1741 and 1668, acetate at 1729 and 1263 and cyclopentanone at 1713 cm^{-1}) and its nmr spectrum (see Experimental Section) which exhibited complex five-proton absorption characteristic of –O–CH– suggested the possibility that a glycoside of a sesquiterpene lactone had been isolated. The nmr spectrum also displayed doublets at 6.16 and 5.14 ppm (exocyclic methylene group) and, after deuterium exchange, a multiplet at 4.78 ppm characteristic of partial formula A, a methyl singlet, a deshielded methyl doublet at 1.34, an acetate singlet, and three hydroxyl protons which disappeared after deuterium exchange.

The presence of three hydroxyl groups was confirmed by conversion to a triacetate. Treatment with dilute acetic acid afforded aromatin (**12**) of known structure



12



13a, R_1 = Ac, R_2, R_3, R_4 = H
b, R_1, R_2, R_4 = H; R_3 = Ac
c, R_1, R_2, R_3 = H; R_4 = Ac
d, R_1, R_2, R_3, R_4 = Ac

and stereochemistry.[27] The facile elimination reaction and the deshielding of the C-10 methyl group suggested that the carbohydrate moiety was attached to C-2 and probably α to the sesquiterpene nucleus.

At this point a report[28] appeared on the isolation from two *Baileya* species (tribe *Helenieae*) of a sesquiterpene glucoside paucin for which formula **13a** or **13b** was proposed. The properties of paucin and our substance from *H. odorata* were sufficiently similar to warrant a direct comparison which established identity of our material with paucin.

The earlier assignment[28] of the acetyl group to C-2′ or C-4′ of the glucose moiety was based on the observation that paucin consumed only 1 mol equiv of HIO_4 when oxidized with a twofold molar excess of the reagent. However, examination of the 100-MHz spectrum of paucin revealed *three doublets* which corresponded to the three hydroxyl groups at 4.64, 4.81, and 4.95 ppm (J = 4.0, 3.8, and 4.8 Hz) because they disappeared on deuterium exchange. Formulae **13a** and **13b** would require two doublets and one triplet. Hence we prefer structure **13c** for paucin.[29] The resonance of the anomeric hydrogen was displayed as a doublet at 4.37 (J = 7.0 Hz); the large coupling constant confirmed that the glycoside moiety in paucin was β.

Hymenoxynin.—The polarity and empirical formula of the crystalline material from the Coahuila collection of *H. odorata* suggested that it, too, was a glycoside but its ir (one γ-lactone band at 1770 cm^{-1}) and nmr spectrum (Experimental Section) showed that the sesquiterpene lactone nucleus differed from that of paucin. More specifically, the presence of two methyl doublets and one methyl singlet required saturation of the exocyclic methylene group. Deuterium exchange resulted in the disappearance of four hydroxyl protons and made evident one H–CO singlet at 4.35, one H–CO doublet at 4.47 (J = 7.5 Hz),[30] one H–CO multiplet at 4.70 associated with H-8, and a plethora of signals corresponding to eight H–CO protons in the region 3.2–4.0 ppm. Acetylation to a tetraacetyl derivative confirmed the presence of four hydroxyl groups; the

(26) For a number of leading references, see W. Herz, P. S. Subramaniam, P. S. Santhanam, K. Aota, and A. L. Hall, *J. Org. Chem.*, **35**, 1453 (1970).

(27) J. Romo, P. Joseph Nathan, and F. Diaz, *Tetrahedron*, **79**, 20 (1964). We wish to thank Dr. Romo for an authentic specimen.

(28) T. G. Waddell and T. A. Geissman, *Tetrahedron Lett.*, 515 (1969). We wish to thank Professor Geissman for an authentic sample of paucin.

(29) Observation of the H-6′ resonance in the nmr spectrum of **13d** which would have resolved the ambiguity was not possible owing to overlapping of the signals.

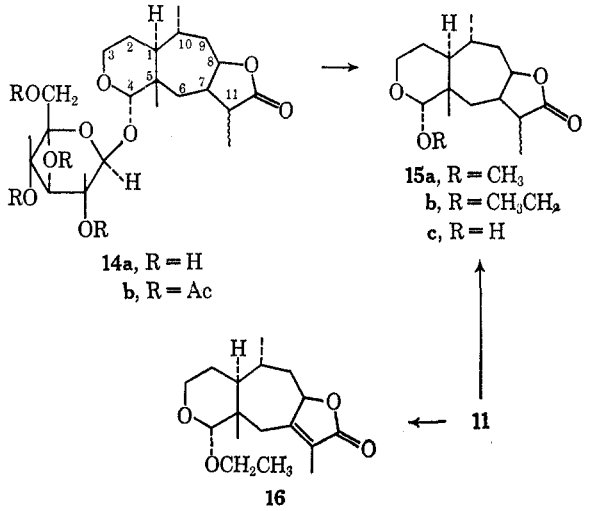
(30) Later assigned to H-4 and H-1′, respectively.

accompanying downfield shift of five protons indicated the presence of one primary hydroxyl, three secondary hydroxyls and apparently three ether hydrogens in addition to the three protons responsible for signals in the region of 4.3–4.7 ppm.

On methanolysis with concentrated hydrochloric acid at room temperature hymenoxynin afforded a methyl ether $C_{16}H_{26}O_4$ (**15a**, nmr spectrum) and glucose which was isolated as the osazone, thus establishing that hymenoxynin was an O-glucoside in which the glucose moiety was attached to the hemiacetal linkage of a sesquiterpene lactone. The nmr spectrum of **15a** had the usual H-8 H–CO multiplet at 4.77, a sharp singlet at 4.04, whose chemical shift and appearance was reminiscent of H-4 in hymenolide, and two broad signals (one proton each) at 3.70 and 2.93 ppm probably associated with CH_2O. Analogously, treatment of hymenoxynin with ethanolic hydrochloric acid afforded the ethoxy derivative **15b** and hydrolysis of hymenoxynin with hydrochloric acid in aqueous acetone yielded a lactol **15c**.

Hymenoxynin could be correlated with hymenolide in the following manner. Hydrogenation of anhydrohymenolide (**11**) with platinum oxide in ethanol afforded dihydroisoanhydrohymenolide (**16**) and a tetrahydro derivative which was identical in all respects with the hymenoxynin derivative **15b**. Since the configuration of hymenolide had been established, as will be discussed subsequently, the remaining uncertainties in the structure of hymenoxynin were the configuration at C-11 and the configuration of the C-1′ anomeric carbon atom.

In the nmr spectrum of hymenoxynin, the signal of the anomeric proton was observed at 4.47 ppm with a large coupling constant (J = 7.5 Hz) characteristic of *trans*-diaxial coupling to H-2′. Hence, the glycosidic linkage of hymenoxynin was β and hymenoxynin could be expressed as (**14a**), where the configuration at C-11 remains to be established.



Stereochemistry.—The compounds listed in Table I could not, with the exception of paucin, be correlated with other substances of known stereochemistry. As a consequence, assignment of configuration to the various asymmetric centers had to depend on more circumstantial evidence.

At the outset, it was logical to assume that the C-7 side chain, as in almost all sesquiterpene lactones of natural origin, was equatorial and β, and that H-1 was α, C-5 methyl β, and C-10 methyl α, as in all other pseudoguaianolides and modified pseudoguaianolides from *Helenium* and *Gaillardia* species. This supposition was strongly reinforced by the discovery that paucin was a derivative of aromatin (**12**) and possessed the postulated stereochemistry at C-1, C-5, C-7, and C-10. Furthermore, the gross structure of hymenolide and hymenoxynin suggested that these constituents of chemical varieties of *H. odorata* were formed by biological oxidation of odoratin,[18] a compound of known stereochemistry which had been isolated previously[15] from a different collection of *H. odorata*.[31] On this basis, vermeerin and floribundin would have structures **2** or **3** and would be C-8 epimers.

Concrete support for these expressions and evidence for the absolute configuration at C-8 was provided by a detailed analysis of the nmr spectra which will now be discussed.[32]

Vermeerin and Floribundin.—The results of our analysis (first order) of spectra obtained at 100 MHz are summarized in Tables II and III. Our analysis of vermeerin corresponds in the main to an interpretation published previously.[21] However, the South African authors incorrectly characterized the multiplets of H-1, H-2a, and H-2b as an ABX system. The 100-MHz spectrum ($CDCl_3$) displays at *ca.* 2.06 ppm a quartet which only partially represents the H-1 multiplet since H-1, being further coupled to H-10, cannot give rise to an X quartet. The distance between the outer lines of this "quartet" is about 13.5 Hz, which corresponds to the $|J_{AX} + J_{AB}|$ referred to by Anderson and coworkers.[21] In view of the relative chemical shifts of H-1, H-2a, and H-2b, and considering the coupling H-1,H-10, the quartet must be the ABC end of a larger spin system which includes H-9a and H-9b as well and cannot be analyzed exactly because of virtual coupling and because of the fact that all multiplets coincide with the multiplets due to other protons.

Line identification by means of tickling experiments showed that the lower field proton H-2a possess first-order splittings $S_{2a,2b} \cong 17.6$ and $S_{1,2a} \cong 5.6$ Hz and the higher field proton H-2b $S_{2a,2b} \cong 17.6$ and $S_{1,2b} \sim 11.5$ Hz. Comparison with the 220-MHz spectrum which provides first-order splittings $S_{2a,2b} = 18.3$, $S_{1,2a} = 5.4$, and $S_{1,2b} = 12.6$ Hz actually indicates that the system still interacts strongly at 100 MHz. These first-order splittings clearly indicate the presence of one large and one smaller vicinal interaction; assuming that both of them have the same sign, the correct coupling constants which would result from an exact analysis

(31) Unfortunately attempts to investigate the transformation of odoratin (i, footnote 13), generously supplied by Dr. Romo de Vivar, into hymenolide or one of the dihydrovermeerins or dihydrofloribundins foundered on the paucity of available starting material. Oxidation by several methods resulted in a complex mixture of products. Hymenolide which is the ethyl hemiacetal of the dialdehyde produced by glycol cleavage of odoratin is quite possibly an artefact produced from the dialdehyde or from a glycoside corresponding to **8a** by reaction with ethanol under the slightly acid conditions employed during the lead acetate precipitation stage.

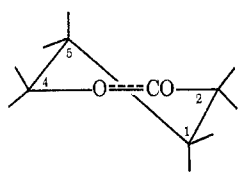
(32) The recent generalization[33] that the sign of the lactone Cotton effect of a sesquiterpene lactone which incorporates partial structure A can be used for assignment of absolute configuration is not applicable to the dilactones listed in Table I because of the superposition of two Cotton effects in the region in question.

(33) T. G. Waddell, W. Stöcklin, and T. A. Geissman, *Tetrahedron Lett.*, 1313 (1969).

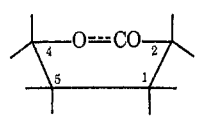
must fulfill the conditions $J_{1,2a} < S_{1,2a}$ and $J_{1,2b} > S_{1,2b}$ (high-field approximation for an ABX system). In the 220-MHz spectrum the H-1 resonance was seen as degenerate octet whose exact analysis was not possible because of virtual line broadening.[34] The approximate first-order splittings were S_1 (corresponding to $J_{1,10}$) = 8.4 Hz, S_2 (corresponding to $J_{1,2a}$) = 5.9 Hz, and S_3 (corresponding to $J_{1,2b}$) = 12.9 Hz.

In view of these and our other results, the following conclusions can be reached about the stereochemistry of vermeerin and floribundin. We will first consider the six-membered lactone ring of vermeerin and floribundin.

X-Ray and ir studies have demonstrated that the carbonyl stretching frequency of a δ-lactone in the half-chair form J lies in the range 1730–1750 and that it is higher, approximately 1758–1765 cm^{-1}, for the half-boat form K.[35] The utility of this rule has been demonstrated recently in the case of some iridolactones.[36] Since the ir spectra of vermeerin and floribundin show ν_{CO} = 1729 and 1727 cm^{-1}, respectively, it can be assumed that the δ-lactone ring of both compounds possesses the "half-chair" conformation. Support for this is found in the detailed analysis of the nmr spectra.

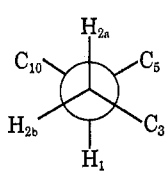


J

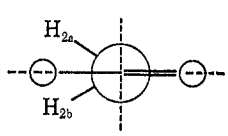


K

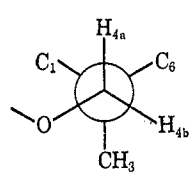
In the spectra of 2 and **3**, $J_{1,2a}$ and $J_{1,2b}$ are nearly the same and chemical shifts of H-2a and H-2b are in the same order. From the magnitude of the J's it follows that the relative configuration of the fragment C-2, C-1 can be expressed approximately by the Newman projection L.



L



M



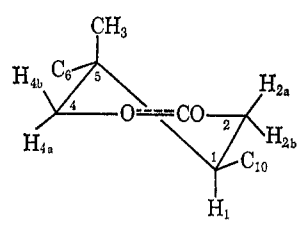
N

Both compounds exhibit a rather large geminal coupling constant $^2J_{2a,2b}$ = |18–19| Hz. Since the protons in question are attached to sp^3, not sp^2, hybridized carbon atoms, the large value must have its origin in hyperconjugative enhancement, by $\sigma-\pi$ interactions, of the order of $J^\pi \sim$ 6–7 Hz.[23,37] Such a value of J^π requires, according to theoretical calculations,[38] that a line joining H-2a and H-2b be perpendicular to the plane of the conjugated system as in M.

Moreover, in 2 and **3**, one of the H-4 protons is coupled to the C-5 methyl group,[39] obviously a coupling of the W-type, which requires that the C–H and C–CH_3 bonds be antiparallel.[23] Hence the fragment C-4, C-5 can be represented approximately by projection N.

In principle, part structures L and N could be accommodated in the half-chair or the half-boat form, but the requirement for hyperconjugative interaction imposed by M is realized in the half-chair form only. In the half-boat form the dihedral angles which the H–C-2 bonds make with the plane of the conjugated CO–O system would not be equal as required by M but would differ considerably from each other, the pseudoequatorial bond lying approximately in the plane. Measurements of the solvent effect (Table IV) further demonstrate that there is little difference in the position of H-2a and H-2b with respect to the plane of the δ-lactone group since values of the solvent shift $\Delta = \delta_{CDCl_3} - \delta_{C_6D_6}$ for H-2a and H-2b are practically identical. Moreover, there is no significant difference between the Δ values for H-2 and H-4 of vermeerin on the one hand and floribundin on the other. This indicates that vermeerin and floribundin form collision complexes with approximately the same geometry.

These observations clearly support the conclusion that the δ-lactone ring is in the half-chair conformation and that the configuration of asymmetric centers C-1 and C-5 is the same in both compounds. Combination of these requirements with L and N leads to expression O for the δ-lactone ring in which H-1 and C-5 methyl are *trans*.[40]



O

We now proceed to consider the stereochemistry of the γ-lactone ring. A solution to this problem could be based, in principle, on the magnitude of the vicinal interaction of the bridgehead protons, although it is possible to distinguish unequivocally between *cis* and *trans* fusion only when conformational rigidity imposes characteristic differences of dihedral angles approximately in the sense of formulas P and Q[42] which are shown below.

(34) H-1 is part of a relatively strongly interacting system involving H-2a, H-2b, H-1, H-10, H-9a, and H-9b,; hence the multiplet couln include combination lines or wings, and the existence of true $\sigma-\sigma$ long range interactions could not be excluded.

(35) K. K. Cheung, K. H. Overton, and G. A. Sim, *Chem. Commun.*, 634 (1965).

(36) K. Sisido, K. Inomata, T. Kageyama, and K. Utimoto, *J. Org. Chem.*, **33**, 3149 (1968).

(37) Methane, J = |12.4| Hz, serves as reference.

(38) VB method, M. Barfield and D. M. Grant, *J. Amer. Chem. Soc.*, **85**, 1899 (1963); MO method, J. A. Pople and A. A. Bothner-By, *J. Chem. Phys.*, **42**, 1339 (1965).

(39) The low-field doublet of H-4a had a 1 Hz larger line width than the high-field doublet of H-4b; the presence of long-range coupling to the methyl group was established by tickling experiments.

(40) Unfortunately, it was not possible to deduce the relative configuration of H-1 and C-5 methyl directly from the nmr spectrum. The presence of $^4J_{1,15}$ which might have served to confirm the *trans* relationship could not be established. However, absence of long-range coupling of this type is not a sufficient criterion for deciding against a *trans*-ring fusion since a ^4J interaction of the angular type can be significantly influenced by the conformation of the molecule as a whole.[41]

(41) N. S. Bhacca, J. E. Gurst, and D. H. Williams, *J. Amer. Chem. Soc.*, **87**, 302 (1965).

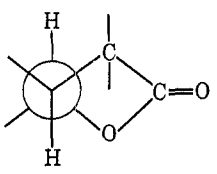
(42) J. T. Pinhey and S. Sternhell, *Aust. J. Chem.*, **18**, 543 (1965).

TABLE IV

SOLVENT SHIFTS OF VERMEERIN AND FLORIBUNDIN[a]

	H-2a	H-2b	H-4a	H-4b	H-7	H-8	H-14	H-15	H-13a	H-13b
2	0.50	0.52	0.75	0.67	0.89	0.84	0.66	0.70	0.12	0.65
3	0.50	0.56	0.73	0.63	0.90	0.70	0.64	0.65	0.11	0.61

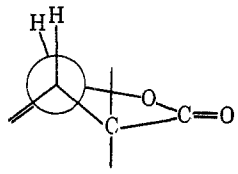
[a] Shifts expressed in $\Delta = \delta_{CDCl_3} - \delta_{C_6D_6}$; values are based on centers of multiplets; measured on Varian HA-100 using hexamethyldisiloxane as internal standard.



trans

$^3J_{H,H} > 10$ Hz

P



cis

$^3J_{H,H} \leqq 8$–9 Hz

Q

This situation usually exists in the case of those systems where a γ-lactone ring is fused onto a six-membered ring. When, however, the γ-lactone is attached to a ring containing more than six carbon atoms, greater flexibility can be expected, and the difference in coupling constants may not be particularly significant.[43]

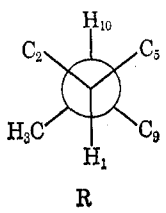
Table III indicates that the $J_{7,8}$'s in vermeerin and floribundin are not sufficiently different to warrant immediate application of the above rule. Nevertheless, comparative studies on a large group of analogous γ-lactones with well-defined stereochemistry, the detailed discussion of which will be published elsewhere,[44] have shown that the rule $0 < S_{7,8}$ (*cis*) < 8–$9 < S_{7,8}$ (*trans*) for the first-order values of $J_{7,8}$ appears to be generally applicable, especially if both isomers are available for comparison. Therefore, we assume that the fusion of the γ-lactone ring in vermeerin is *trans* and in floribundin *cis*.

This assumption is further supported by considering the magnitude of the allylic couplings of H-13a and H-13b with H-7. It has been found, by analyzing a large number of naturally occurring sesquiterpene lactones of well-defined stereochemistry containing six-, seven-, and ten-membered rings, that $^4J_{cis} \leqq 3$ and $^4J_{trans} \geqq 3$ Hz.[45] The application of this rule to the case at hand (Table II) again suggests that vermeerin is the *trans*- and floribundin the *cis*-fused isomer.

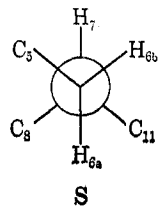
With a knowledge of the stereochemistry of the two lactone rings in vermeerin and floribundin, it should be possible in principle to establish the relative configuration of all asymmetric centers, using Dreiding models and the data of Tables II and III. However, models do not necessarily reflect the actual geometry of the molecules in question.[46] Secondly, the data obtained from first-order analysis of the nmr spectra do not always represent the true coupling constants and are incomplete with respect to the vicinal couplings H-9, H-10 which could not be estimated owing to superposition of signals. In solving the conformational problem of the seven-membered ring, the following additional data are of importance.

(1) Coupling $J_{1,10}$: The magnitude of $J_{1,10}$ determines the relative configuration at C-1,C-10; hence, if the configuration of H-1 is established, the configuration at C-10 is known. In the case of floribundin, $J_{1,10} = 11.4$ Hz and the diaxial relationship of H-1 and H-10 shown in R can be deduced. In the case of vermeerin, $J_{1,10} = 8.4$ Hz, a value which unfortunately does not permit an unequivocal decision as to the nature of the H-1,H-10 relationship, although it seems highly unlikely that the stereochemistry at C-10 is different from that in floribundin.

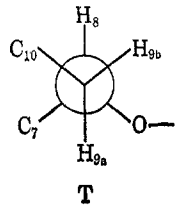
(2) Couplings $J_{6,7}$ and $J_{8,9}$: Table III clearly indicates the presence of one diaxial coupling around the bonds C-6,C-7 and C-8,C-9 of both compounds, as illustrated in S and T.[48]



R



S



T

If we assume a half-chair conformation for the δ-lactone ring and a *trans* fusion of the δ-lactone with the seven-membered ring (O) as deduced previously, a limited number of possible arrangements containing a *cis*- or *trans*-γ-lactone-ring fusion exists. These are summarized in Table V.

Let us consider first the floribundin molecule. Two formulas, **3** and **3a**, which express the relative stereochemistry, can be written for each of which there exists only one suitable conformation. In the case of **3**, this is the conformation containing the C_1[49] (or C_6) boat form of the seven-membered ring; in the case of **3a**, it is the C_1 chair form. Both conformations are in accord with the data presented up to this point. A distinction between the two would be possible if the vicinal couplings around the C-9,C-10 bond could be estimated. The C_1 boat form of **3** would be preferred if one assumes that repulsion due to the C-5 methyl group exerts a dominant influence. Its model indicates that it contains only two 1,3-diaxial interactions, $CH_3 \longleftrightarrow$ H-2a and $CH_3 \longleftrightarrow$ H-10, whereas the C_1 chair of **3b** also contains the repulsion $CH_3 \longleftrightarrow$ H-7. On the other hand, the C_1 chair form of **3a** contains only one transannular H–H interaction of importance, H-9 (*endo*) $\longleftrightarrow$ H-6 (*endo*),

(43) For example, in compounds of the santonin series, $^3J_{trans} = 11$–12 and $^3J_{cis} = 8$ Hz. On the other hand, in isophotosantoinic acid lactone (*trans*), $J_{6,7} = 9.0$ Hz, and, in 6-epiisophotosantonic acid lactone (*cis*), $J_{6,7} = 7.8$ Hz.[42]

(44) Z. Samek, in preparation. Because first-order values can be used only within certain ranges of $|J/\Delta\nu|$, it may be necessary to vary internal shifts by means of solvent effects or by change of external field.

(45) Z. Samek, *Tetrahedron Lett.*, 671 (1970).

(46) For example, in the case of bromohelenalin, significant differences between the structure determined by X-ray analysis[47] and the model were found.

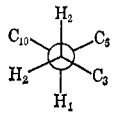
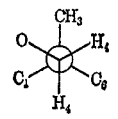
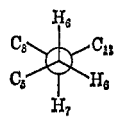
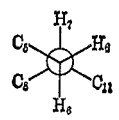
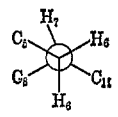
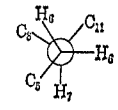
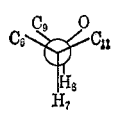
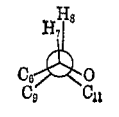
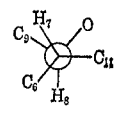
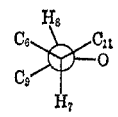
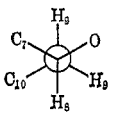
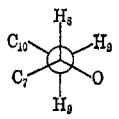
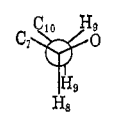
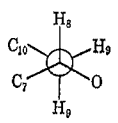
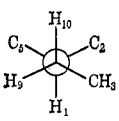
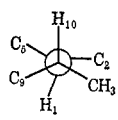
(47) M. T. Emerson, C. N. Caughlan, and W. Herz, *Tetrahedron Lett.*, 621 (1964); Mazhar-ul-Haque and C. N. Caughlan, *J. Chem. Soc. B*, 956 (1969).

(48) It should be noted that the internal shifts between "pseudoaxial" and "pseudoequatorial" protons on both bonds of floribundin have opposite signs when compared with corresponding internal shifts in the vermeerin spectrum.

(49) The symbol C_n refers to the atom lying in the plane of symmetry of the nondistorted boat or chair form of the cycloheptane ring.

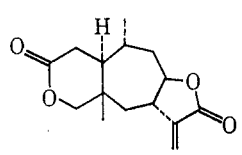
TABLE V

CONFORMATIONS OF VERMEERIN AND FLORIBUNDIN

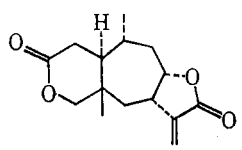
Fragment	C_1 boat[a] 1α, 5β, 7H-α, 8H-α	C_1 chair[b] 1α, 5β, 7H-β, 8H-β	C_5 chair[c] 1α, 5β, 7H-β, 8H-α	C_9 chair[d,e] 1α, 5β, 7H-α, 8H-β
C_2–C_1		*f*	*f*	*f*
C_4–C_5		*f*	*f*	*f*
C_6–C_7				
C_7–C_8				
C_8–C_9				
C_{10}–C_1		*f*		*f*

[a] Flexible, slightly folded. [b] Fixed by nonbonded interactions; seven-membered ring possesses normal, nondistorted conformation of "free" cycloheptane ring. [c] Flexible, interconvertible by twisting with C_6 chair which is excluded for steric reasons. [d] Fixed by transannular interaction of H_1 and H_2. [e] Twisted C_8 boat or C_6 flattened boat excluded by couplings H_6–H_7. [f] Conformation identical with that given in preceding column.

whereas in the C_1 boat of **3** there are the repulsions H-1 ⟷ H-7, H-8 and H-6 ⟷ H-10, H-9 (*endo*) to be considered.



2a

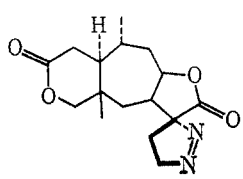


3a

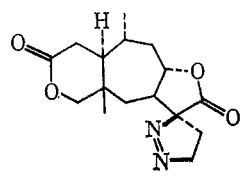
For vermeerin there are also only two possibilities with suitable conformations of the seven-membered ring, the C_5 chair form containing a pseudoequatorial C-10 methyl group and the relative stereochemistry of **2a**, and the C_9 chair form with the relative stereochemistry of **2**,[50] but in the C_5 chair form of **2a** the substituents on the C-8,C-9 bond are eclipsed which contradicts the observed splittings corresponding to $J_{8,9a}$ and $J_{8,9b}$ (Table III). Moreover, in this form the substituents on C-8,C-9 are staggered. This would result in strong puckering of the γ-lactone ring and affect the resonance stabilization adversely. Hence the C_9 chair of formula **2** is preferred. The decreased dihedral angles between H-1 and H-10 in this conformation explain the smaller value of $J_{1,10}$ in vermeerin.

The configurations of vermeerin and floribundin which have been deduced in the foregoing sections are supported by the CD curves of their pyrazolines. Inspection of the models shows that formation of a pyrazoline by reaction of floribundin (**3**) with diazomethane should occur predominantly, if not exclusively, from the α side to give **17**. On the basis of a recently deduced relationship[51] between the absolute configuration of such pyrazolines and the sign of their Cotton effect in the 330-nm region, one would expect a strongly positive CD curve for **17**. This was indeed observed ($[\theta]_{319}$ = 11,800).

The negative CD curve of the pyrazoline of vermeerin ($[\theta]_{324.5}$ = −10,000) indicates configuration **18**, formed by approach from the β side. Inspection of the model suggests that the β face of **2** (in the conformation deduced previously) is probably more accessible, but not so unambiguously so, as the α face of **3**.



17



18

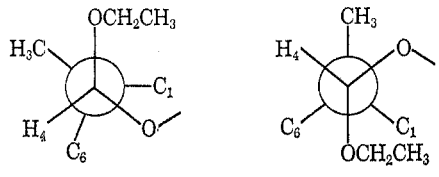
Hymenolide.—Comparison of the nmr spectrum (Tables II and III) of anhydrohymenolide (**10**) with the spectrum of floribundin suggested that the two compounds, and therefore hymenolide also, possessed the same stereochemistry at the common asymmetric cen-

(50) As noted previously, the magnitude of $J_{1,10}$ in vermeerin does not permit unequivocal determination of the configuration at C-10. However, leaving aside biogenetic arguments, comparison of Dreiding models of **2** and **2a** with models of their C-10 epimers indicates that the β configuration of the C-10 methyl group induces repulsions which makes this configuration inherently less probable.

(51) G. Snatzke, *Riechst., Aromen, Körperpflegem.*, **19**, 98 (1969); M. Suchy, L. Dolejs, V. Herout, V. Sorm, G. Snatzke, and J. Himmelreich, *Collect. Czech. Chem. Commun.*, **34**, 229 (1969).

ters C-1, C-5, C-7, C-8, and C-10. This was verified by the chemical correlation of **10** with themoidin (*vide supra*).

The probable configuration of **10** at C-4 could be deduced by taking into consideration the long-range couplings exhibited by the signal of H-4. Decoupling experiments revealed the absence of H-4,H-15 coupling, but it could be clearly demonstrated that $J_{3,4} \neq 0$. In order to account for this long-range coupling by the W path, H-4 should be pseudoequatorial as in U (six-membered ring, a half-boat) or in V (six-membered ring, a half-chair). Because of the presence of torsional strain in U, V (with H-4β) seemed more probable than U. This inference was also in accord with the indication that the δ-lactone ring of **9** is probably in the half-boat form (ir band of δ lactone superimposed on ir band of γ lactone at 1756 cm^{-1}), possibly because this allows the ethoxy group to occupy a pseudoequatorial position when the anomeric effect is minimized by introduction of the carbonyl group at C-3.



U V

The appearance of the H-3 signal of hymenolide as a doublet of doublets with $J_{2a,3} = 10.0$ and $J_{2b,3} = 2.5$ Hz (10.0 and 3.0 Hz in the case of **8b**) indicated that the C-3 hydroxyl group was equatorial and β. Hence, both oxygen functions on the hemiacetal ring of hymenolide are in the stable orientation, the C-3 hydroxyl group equatorial (β) and the C-4 ethoxy group axial and α (anomeric effect) as in **8a**. Furthermore, although the method of correlation of hymenoxynin with **8a** allowed for possible epimerization of hymenoxynin at C-4, the relative constancy in the chemical shift of H-4 and the presumed greater stability of an α-oriented acetal linkage at C-4 argue in favor of the proposition that the configuration of hymenoxynin at C-4 is the same as that of hymenolide, *i.e.*, as depicted in **14a**.

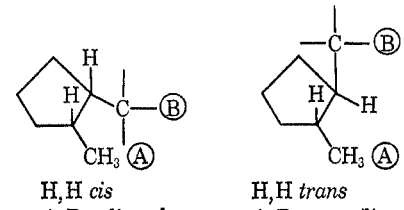
Greenein.—The infrared spectrum of greenein indicated that the δ-lactone ring (ir band at 1735 cm^{-1}) was in the half-chair form. Conformational analysis of the system in the manner described for vermeerin and floribundin was unfortunately not possible because the coupling constants $J_{1,2}$, $J_{1,10}$, and $J_{1,15}$ could not be determined and $J_{4,15}$, so important to establishing the nature of the ring junction in **2** and **3**, was necessarily absent. However, the close correspondence of the nmr spectra of greenein, **3** and **10** (see Tables II and III), indicated that the conformation of the seven-membered ring and the γ-lactone ring was probably the same.

Anthemoidin and Themoidin.—Anthemoidin (**4b**) and themoidin (**5a**) were identified as dihydro derivatives of vermeerin and floribundin, respectively. Two other dihydro derivatives were also encountered, 11-epianthemoidin (dihydrovermeerin A, **4a**) and 11-epithemoidin (**5b**).[52]

(52) **4a** was the only compound isolated when vermeerin was reduced with sodium amalgam–acetic acid. It was stable to base, as expected. Consequently, we were somewhat puzzled to find that themoidin, isolated in excellent yield by sodium amalgam reduction of floribundin, was isomerized by base treatment to a C-11 epimer **5b**. Obviously, in this instance, catalytic *and* sodium amalgam reduction afforded the less stable C-11 epimer.

The problem of solving the configuration of anthemoidin at C-11 relative to C-7 was solved by determining the magnitude of the coupling constant $J_{7,11}$.[42,53] The relative magnitudes of $J_{7,11}$ for **4a** (12.7 Hz) and **4b** (7.5 Hz) indicated that H-7 and H-11 are *cis* in anthemoidin (**4b**) and *trans* in its C-11 epimer (**4a**). The situation is not so simple in the case of themoidin and its C-11 epimer. We find $J_{7,11}$ for themoidin in the range of 8–9 Hz, an intermediate value (possibly due to greater flexibility of the *cis*-γ-lactone ring) which permits no clear decision regarding the steric relationship between H-7 and H-11. Moreover, the value of $J_{7,11}$ for 11-epithemoidin could not be determined accurately owing to overlapping of signals.

An alternative method for establishing the configuration at C-11 is based on the rule[54] that $^3J_{CH_3,H}$ for A,B *trans* < $^3J_{CH_3,H}$ for A,B *cis* (structure I), which apparently holds when the ring is five membered or smaller. The rule seems to be applicable to γ-lactones.[55] Comparison of the H-11, H-13 coupling constants of anthemoidin (6.6 Hz) and its C-11 epimer (7.5 Hz) leads to the same conclusion as before, *i.e.*, H-7 and H-11 *cis* for **4b** and *trans* for **4a**, but, while $J_{11,13}$ for themoidin was 7.3 Hz, the overlap, in the nmr spectrum of 11-epithemoidin, of the H-13 doublet and the H-6 multiplet interfered with the determination of $J_{11,13}$[56] and the application of the above method to determining the C-11 stereochemistry of themoidin.



H,H *cis* A,B eclipsed H,H *trans* A,B not eclipsed

Tentative assignment of formula **5a** to themoidin and **5b** to 11-epithemoidin is based on the relative chemical shift of H-11 in the two compounds. On the basis of a discussion of the conformation of saturated γ-lactones by Narayanan and Venkatasubramaniam[53] we suggest that H-11 is not affected significantly by the stereochemistry of the lactone ring *per se* and that its chemical shift should therefore be influenced primarily by the relative position of H-11 with respect to the shielding effects produced by the nearest anisotropic groups, *i.e.*, the bonds C-A,C-7 and C-7,C-B and the lactone group. However, it would be difficult to assess the separate contributions of these groups to the chemical shift of H-11 and to arrive at an *a priori* prediction of the sign of $\delta_{H\text{-}11\alpha} - \delta_{H\text{-}11\beta}$ which, granting the above argument, should remain constant.

While the literature does not contain many detailed analyses of nmr spectra of compounds with partial structure W, examination of the few examples which can be brought to bear on the problem (Table VI) sup-

(53) C. R. Narayanan and N. K. Venkatasubramaniam, *J. Org. Chem.*, **33**, 3156 (1968).

(54) J. Wolinsky, T. Gibson, D. Chan, and H. Wolf, *Tetrahedron*, **21**, 1247 (1965).

(55) Z. Samek, manuscript in preparation. Accurate measurement is necessary since the difference in $C_{11,13}$ coupling constants within a set of epimers is generally small.

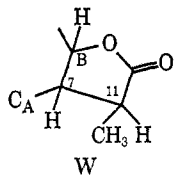
(56) In measuring INDOR spectra of H-13, signals of H-7 and H-11 change intensities since H-7 and H-11 overlap also and the estimation of $||^3J_{11,13} + {}^3J_{11,7}||$ becomes exceedingly difficult.

TABLE VI
CHEMICAL SHIFTS OF H-11

Compd	Stereochemistry	δ_{H-11}[a]
α-Santonin	H-6β, H-7α, H-11β	2.5[b]
β-Santonin	H-6β, H-7α, H-11α	2.76[b]
6-*epi*-α-Santonin	H-6α, H-7α, H-11β	2.55[b]
6-*epi*-β-Santonin	H-6α, H-7α, H-11α	2.92[b]
11-*epi*-Anthemoidin	H-7α, H-8β, H-11β	2.22[c]
Anthemoidin	H-7α, H-8β, H-11α	2.60[c]

[a] Ppm. [b] Reference 42. [c] This work.

ports the above assumption and leads to the tentative rule δ_{H-11} (H-7,H-11 *cis*) > δ_{H-11} (H-7,H-11 *trans*).[57] On this basis, themoidin (δ_{H-11} 2.92) is represented by formula **5a** and its C-11 epimer (δ_{H-11} 2.2) by **5b**.



W

Experimental Section[58]

Extraction of *Hymenoxys richardsonii* (Hook) Ckll. var. *floribunda* (Gray) Parker.—Above-ground material, wt 2.7 kg, collected by Dr. B. H. Braun in July 1962, in the vicinity of Boulder, Colo., was extracted with chloroform in the usual fashion.[59] The crude gum was chromatographed over 600 g of silicic acid, 800-ml fractions being collected. Fractions 1–15 (Bz, Bz–Chlf 3:1) eluted 2.4 g of oil, fractions 16–17 (Bz–Chlf 2:1) eluted 1.35 g of gum, and fractions 18–21 (Bz–Chlf 2:1) eluted 7.4 g of crystalline material. Recrystallization from Chlf–ether afforded vermeerin (**2**), mp 145–146°, $[\alpha]_D$ −67.1° (*c* 0.394), which had ir bands of 1766, 1729, and 1670 cm^{-1}, identical in all respects with authentic vermeerin. Vermeerin was not affected by treatment with hot acetic acid or boron trifluoride etherate.

Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63; O, 24.21. Found: C, 68.08; H, 7.57; O, 24.50.

Fractions 22–34 (Bz–Chlf 2:1 and 1:1) yielded 6.6 g of crystalline material. Recrystallization from acetone–ether afforded floribundin (**3**) which had mp 143°; $[\alpha]_D$ +84.0° (*c* 0.319); ir bands at 1757, 1727, and 1656 cm^{-1}; uv absorption λ_{max} 212.5 nm (ε 8300).

Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63; O, 24.21. Found: C, 68.19; H, 7.81; O, 24.69.

Fractions 76–82 (Bz–Chlf 1:3) afforded 0.85 g of yellow solid. Recrystallization from dioxane–ethyl acetate gave hispidulin (**1**) mp 290–293°, identical in all respects with authentic material from *Ambrosia hispida*. Further elution with Bz–Chlf or more polar solvents gave 25.1 g of gummy mixtures (tlc) which could not be separated satisfactorily.

The crude gum from 25 kg of *H. richardsonii* var. *floribunda*, collected by Mr. R. J. Barr on July 17, 1968, 10 miles southeast of Springerville, Apache County, Ariz., at 7500-ft elevation (Barr No. 68363, on deposit in herbarium of Florida State University) weighed 685 g. A 280-g portion was chromatographed over 2.6 kg of silicic acid in the usual way. Fractions 1–8 (Bz, Bz–Chlf 1:2) gave 7.0 g of gum and fractions 9–38 (Bz–Chlf 1:2 to Chlf) gave 23.6 g of crude vermeerin. Fractions 39–60 (Chlf to Chlf–MeOH 93:7) gave 121 g of a gum which contained several compounds including apparently some floribundin (tlc). Successive elution with Chlf–MeOH (93:7, fractions 61–62) gave 90 g of gummy mixture. Efforts to separate the constituents of these fractions are under way. Fractions 63–67 (Chlf–MeOH 93:7) gave a yellow solid which on recrystallization afforded 2.47 g of hispidulin. Further elution with methanol gave a gummy mixture.

Pyrazoline of Vermeerin—Vermeerin, wt 136 mg, in 40 ml of anhydrous ether and 1 ml of methanol was mixed with excess diazomethane in ether and left in the refrigerator for 4 days. Evaporation at reduced pressure afforded 142 mg of solid which was recrystallized from ethyl acetate. The product had mp 125–126° dec, ir bands at 1776 and 1730 cm^{-1}, CD curve (*c* 0.7 mg/30 ml), $[\theta]_{324.5}$ −10,000.

Anal. Calcd for $C_{16}H_{22}N_2O_4$: C, 62.73; H, 7.24. Found: C, 62.73; H, 7.34.

Pyrazoline of Floribundin.—Treatment of 220 mg of floribundin with diazomethane in the manner described in the previous paragraph gave 243 mg of crude pyrazoline. Recrystallization from ethyl acetate–chloroform yielded colorless needles which had mp 134–135° dec, ir bands at 1775 and 1735 cm^{-1}, CD curve (*c* 1.18 mg/25 ml), $[\theta]_{319}$ +11,800.

Anal. Calcd for $C_{16}H_{22}N_2O_4$: C, 62.73; H, 7.24; N, 9.14. Found: C, 63.27; H, 7.07; N, 8.80.

Reduction of Vermeerin. (A).—A solution of 601 mg of vermeerin in 10 ml of acetic acid was reduced in a hydrogen atmosphere with 71 mg of platinum oxide for 1.5 hr, and was filtered and evaporated. The residue was taken up in dichloromethane, washed and evaporated, and the crude product, wt 0.6 g, chromatographed over 120 g of silica gel, 80-ml fractions being collected. Fractions 1–10 (Bz–ether 17:3) eluted nothing, fractions 11–89 (same eluent) afforded 382 mg of solid dihydrovermeerin A (**4a**) which was recrystallized from acetone–ether and then had mp 128°, $[\alpha]_D$ −25.7° (*c* 0.247); ir bands at 1774 and 1732 cm^{-1}; mol wt 266 (mass spectrometry). It was recovered unchanged after heating for 1 hr with sodium methoxide in methanol and subsequent acidification.

Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.65; H, 8.33; O, 24.03. Found: C, 67.79; H, 8.38; O, 23.67.

Continued elution with Bz–ether (17:3) gave, in fractions 90–137, 45 mg of a solid mixture of dihydrovermeerin A and B and a third substance C, and, in fractions 138–220, 150 mg of a mixture of dihydrovermeerin B and the third substance C, which was rechromatographed over 40 g of silica gel (40-ml fractions eluent Bz–ether 4:1). Fractions 121–145 eluted 86 mg of dihydrovermeerin B (**4b**) which was recrystallized from acetone–ether and had mp 213–214°. Direct comparison established identity with anthemoidin from *Hymenoxys anthemoides*. Fractions 180–235 eluted 62 mg of the third substance which was recrystallized from acetone–ether and had mp 177–179°, ir bands at 1767 and 1735 cm^{-1}, mol wt 266 (mass spectrometry). Because of the small quantity available, the nmr spectrum could not be determined. We are unable to account for the formation of a third substance but the small amount available prevented further investigation of the apparent discrepancy.

Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.65; H, 8.33. Found: C, 67.78; H, 8.27.

(**B**).—To a solution of 100 mg of vermeerin in 7 ml of ethanol and 0.1 ml of acetic acid was added in small portions 2.5 g of 3% sodium amalgam. After 3 hr, the mixture was separated from mercury, filtered, and evaporated *in vacuo*. The residue was taken up in chloroform, washed, dried, and evaporated. The residue, wt 0.1 g, melted at 123–124° after recrystallization from acetone–ether and was identical with dihydrovermeerin A in all respects.

Reduction of Floribundin. (A).—A solution of 2.0 g of floribundin in 130 ml of ethanol and 1 ml of acetic acid was stirred with 57 g of 3% sodium amalgam for 3 hr and worked up as described in the preceding paragraph. The crude product, wt 2.0 g (two major spots on tlc), was recrystallized from acetone–ether to give 1.15 g of a dihydroderivative [dihydrofloribundin A (**5**)] which had mp 213° and was identical in all respects with themoidin isolated from *H. anthemoides*. The mother liquors contained

(57) Z. Samek and W. Herz, unpublished work. The validity of this rule is presently under investigation; the results will be the subject of a futnre communication.

(58) Melting points are uncorrected. Rotations were run in methanol unless otherwise specified, ultraviolet spectra in 95% ethanol on a Cary Model recording spectrophotometer, infrared spectra in chloroform unless otherwise specified on a Perkin-Elmer Model 257 grating spectrometer, CD curves in methanol on a Jasco ORD/UV-5 recording spectrometer, mass spectra on a Nuclide 12-in. medium resolution mass spectrometer, and routine nmr spectra on a Varian A-60 spectrometer in deuteriochloroform solution with tetramethylsilane serving as internal standard. Analyses were performed in the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences or by Dr. F. Pascher, Bonn, Germany. Silicic acid was Mallinckrodt 100 mesh; petroleum ether was low boiling (30–60°). Chromatographic fractions were routinely monitored and products were checked for purity by tlc on microslides coated with silica gel G. Spots were detected by spraying with concentrated sulfuric acid followed by heating. The following abbreviations are used for chromatographic separations: Bz, benzene; Chlf, chloroform; MeOH, methanol.

(59) W. Herz and G. Högenauer, *J. Org. Chem.*, **27**, 905 (1962).

a mixture of themoidin and other products which could not be separated satisfactorily by chromatography.

(B).—Catalytic hydrogenation of 70 mg of floribundin in 40 ml of acetic acid with 100 mg of platinum oxide at 30 lb/in.^{2} and work-up in the usual way gave a single spot on tlc. Recrystallization from acetone–ether yielded 53 mg of themoidin, mp 212–214°. Similarly, hydrogenation of 55 mg of floribundin (ethanol, palladium on charcoal) gave a gum (single spot on tlc) which was chromatographed over 12 g of silicic acid. Elution with benzene–wet ether (19:1) afforded a solid (single spot on tlc) which after recrystallization gave 43 mg of themoidin, mp 214–215°.

Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.65; H, 8.33; O, 24.03. Found: C, 67.56; H, 8.30; O, 24.62.

11-Epithemoidin (5b).—A solution of 0.5 g of themoidin in 5 ml of methanol containing 0.05 g of sodium was heated for 1 hr, cooled, diluted with water, acidified with dilute sulfuric acid, and extracted with chloroform. The washed and dried organic layer was evaporated and the residual solid (1:3 mixture of **5a** and **5b**) was purified by preparative tlc (silica gel–ether). Recrystallization from acetone–ether afforded 0.28 g of 11-epithemoidin which had mp 146–147°; $[\alpha]D$ +54.1° ($CHCl_3$, *c* 1.02); ir bands at 1762 and 1732 cm^{-1}; nmr signals at 4.36 m (H-8), 3.92 (AB system, line separation 11.5 Hz, H-4a and H-4b), 1.31 d (J = 7.5 Hz), and 1.08 d (J = 7.5 Hz, C-10 and C-11 methyl), and 1.08 ppm (C-5 methyl).

Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.65; H, 8.33; O, 24.03. Found: C, 68.32; H, 8.02; O, 23.94.

Extraction of *Hymenoxys anthemoides* (Juss.) Cass.—Above-ground material, wt 3.6 kg, collected by Mr. P. R. Legnamé and Mr. A. R. Cuezzo on Dec 23, 1965, 4 miles from Santa Rosa at the junction of the road to Bella Vista and on Oct 24, 1966, at kilometer 42 along the road from Santa Rosa to Leales, Department of Leales, Tucuman Province, Argentina (Legnamé and Cuezzo No. 5502 and 5595 on deposit in herbarium of Instituto Miguel Lillo, Tucuman, Argentina), was extracted in the usual manner. The crude gum, wt 64 g, was taken up in 100 ml of benzene and chromatographed over 800 g of silicic acid, 800-ml fractions being collected. Fractions 1–35 (Bz or Bz–Chlf 3:1) eluted nothing and 4.6 g of oil. Fractions 36–45 (Bz–Chlf 3:1) eluted 7.5 g of solid material which after recrystallization from acetone–ether melted at 145–146° and was identified as vermeerin. Fractions 46–58 (Bz–Chlf 3:1) gave 5.1 g of solid which after recrystallization melted at 143° and was identified as floribundin. Fractions 59–70 (Bz–Chlf 3:1) eluted 2.95 g of a mixture of anthemoidin and themoidin (*vide infra*). Fractions 71–142 (Bz–Chlf 2:1, 1:1, 1:2, and 1:3) gave 4.8 g of gum. Fractions 143–169 (Chlf) eluted a trace of gum. Fractions 170–234 (Chlf–MeOH 99:1 to MeOH) eluted 7.35 g of gum.

Rechromatography of the material from fractions 59–70 over 100 g of silicic acid (5-ml fractions of benzene containing increasing proportions of ether) gave in fractions 43–75 (Bz–ether 5:1) 50 mg of anthemoidin which had mp 220–221° after recrystallization from chloroform–ether; $[\alpha]D$ −115.5° (*c* 0.139); ir bands at 1780 and 1735 cm^{-1}; mol wt 266 (mass spectrometry). It was subsequently shown to be identical with dihydrovermeerin B.

Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.65; H, 8.33. Found: C, 67.68; H, 8.38.

Fractions 76–82 (5:1) eluted nothing, fractions 83–139 (5:1) eluted 170 mg of mixture, fractions 140–159 (5:1) eluted 25 mg of themoidin which had mp 219–220° (decreasing to 213–214° on storage) after recrystallization from acetone–ether; $[\alpha]D$ +61.8° ($CHCl_3$, *c* 0.55); mol wt 266; ir bands 1767 and 1731 cm^{-1}. This substance was subsequently shown to be identical with dihydrofloribundin.

Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.65; H, 8.33; O, 24.03. Found: C, 67.61; H, 8.27; O, 23.76.

Extraction of *Hymenoxys greenei* (Ckll.) Rydb.—Above-ground plant, wt 3.1 kg, collected by Dr. H. F. L. Rock on July 5, 1960, on Arizona State Route 65, 42 miles south of Winslow (Rock No. 1104, on deposit in herbarium of Vanderbilt University) was extracted in the usual manner, yield of crude gum 65 g. A 45-g portion was chromatographed over 450 g of aluminum (500-ml fractions), but none of the eluates gave solid. The gummy material eluted with Bz, Bz–Chlf, and Chlf (wt 22 g) was rechromatographed over 450 g of silicic acid (1000-ml fractions). None of the fractions crystallized, and only the material from fractions 53–56 (Bz–Chlf 1:3) and 63–74 (Bz–Chlf 1:3) and 75–82 (Bz–Chlf 1:3) appeared to be reasonably homogeneous (tlc).

Rechromatography of the gum from fractions 53–56 over 60 g of silicic acid gave, in the fraction eluted with benzene–wet ether (19:1), a solid which was recrystallized from acetone–ether to give 220 mg of greenein (**6**): mp 169–171°; ir bands at 1770, 1735, and 1660 cm^{-1}. Rechromatography over silicic acid and elution with Bz–Chlf (3:1) raised the melting point to 175–176°, $[\alpha]D$ +114° (*c* 0.2845).

Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.09; H, 7.59.

Rechromatography of the gum from fractions 63–74 over 170 g of silicic acid gave in the fraction eluted with benzene–wet ether (7:3), a solid which was recrystallized from acetone–ether to give 180 mg of floribundin, mp 142–144°, identical in all respects with the material isolated from *H. richardsonii* var. *floribunda*. Rechromatography of the gum from fraction 75–82 over 60 g of silicic acid gave, in the fraction eluted with benzene–wet ether (100:3), a gum which partially solidified on trituration with ether–hexane (1:1). Recrystallization from acetone–ether afforded 15 mg of an unknown solid, mp 244–246° after recrystallization from acetone–ether.

Hydrogenation of Greenein.—A solution of 40 mg of greenein in 25 ml of ethanol was hydrogenated at 30 lb/in.^{2} with 50 mg of palladium on charcoal. The usual work-up gave a gum which was chromatographed over 10 g of silicic acid. Elution with benzene–wet ether (19:1) gave a solid which was recrystallized from acetone–ether–hexane and had mp 103–105°; ir bands at 1770 and 1735 cm^{-1}; nmr signals a 4.7 m (H-8), 4.35 m (2 protons, H-3), 1.30 (C-5 methyl), and 1.15 d (C-10 and C-11 methyls).

Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.65; H, 8.33. Found: C, 68.06; H, 8.55.

Extraction of *Hymenoxys odorata* DC. (A).—Above-ground material, wt 3.6 kg, collected by Mr. J. L. Strother in July 1965 on Route 54, 17 miles south of Saltillo, Coahuila, Mexico (Strother No. 451 on deposit in herbarium of University of Texas at Austin), was extracted with chloroform in the usual manner. The crude gum, wt 69 g, was taken up in 100 ml of benzene and chromatographed over 900 g of silicic acid (800-ml fractions). Fractions 1–26 (Bz to Bz–Chlf 3:1) eluted nothing. Fractions 27–247 (Bz–Chlf 3:1 to Chlf–MeOH 97:3) gave 47.5 g of gum. Fractions 246–250 (Chlf–MeOH 97:3) eluted 1.85 g of solid material. Recrystallization from acetone afforded 1.2 g of hymenoxynin as colorless needles: mp 125–128°; $[\alpha]D$ −37.6° (pyridine, *c* 0.93); ir bands at 3420 (very strong) and 1770 cm^{-1}; nmr signals (100 MHz, $CDCl_3$–DMSO-d_6 + DOAC) 4.70 m (H-8), 4.47 d (J = 7.5 Hz, α-anomeric H of glucose), 4.35 (H-4), 3.2–4.05 m (8 H, H-2, H-2′, H-3′, H-4′, H-5′, and H-6′ of glucose residue), 1.11 d (J = 6.5 Hz), and 1.05 d (J = 6 Hz, C-10 and C-11 methyls), 0.99 ppm (C-5 methyl).

Anal. Calcd for $C_{21}H_{34}O_9 \cdot H_2O$: C, 56.24; H, 8.09; O, 35.67. Found: C, 56.21; H, 8.06; O, 35.99.

(B).—Above-ground material, wt 22.5 kg, collected by Mr. R. J. Barr on June 16–23, 1968, 1 mile east of Rodeo, Hidalgo County, N. M. (Barr No. 68307 on deposit in herbarium of Florida State University), was extracted in the usual manner. The yield of crude gum was 450 g. A 250-g portion was taken up in benzene and chromatographed over 2.5 kg of silicic acid (800-ml fractions). Fractions 1–29 (Bz and Bz–Chlf) eluted 15.6 g of oily material. Fractions 30–85 (Bz–Chlf 2:1 and 1:1) gave 65 g of gum containing hymenolide (*vide infra*). Further elution with Bz–Chlf 1:1 to Chlf–MeOH 97:3 (fractions 86–142) gave 120 g of gum. Fractions 143–150 (Chlf–MeOH 97:3) gave a yellow solid which was recrystallized from dioxane–ethyl acetate to give yellow needles, mp 290–293° dec, wt 0.298, which were identified as hispidulin by comparison with authentic material and conversion to hispidulin triacetate, mp 168–170°. Fractions 151–163 (Chlf–MeOH 9:1) gave 58 g of gum. Further elution with the same solvent (fractions 164–166) gave solid material, wt 3.2 g, which was recrystallized from methanol and then melted at 177–179°: $[\alpha]D$ +64.4° (pyridine, *c* 0.9); ir bands at 3600–3200 (hydroxyl), 1750, 1660 (conjugated γ-lactone), 1738 (cyclopentanone), and 1720 cm^{-1} (acetate); nmr signals (100 MHz, $CDCl_3$–DMSO-d_6) 6.16 d (J = 2.5 Hz) and 5.14 d (J = 2.0 Hz, exocyclic methylene), 4.95 d (4.0), 4.81 d (J = 3.8 Hz), and 4.64 d (J = 4.0 Hz, hydroxyl protons in glucose residue, disappeared on exchange with deuterioacetic acid), 4.37 (J = 7.0 Hz, H-1), 3.50 (2 protons, CH_2OAc), 2.03 (acetate), 1.34 d (J = 6 Hz, C-10 methyl), and 0.94 ppm (C-5 methyl). This substance was identified as paucin by direct comparison with an authentic sample and by its reactions (*vide infra*). Further elu-

tion (Chlf–MeOH 9:1 to MeOH) provided 6.8 g of gummy material.

Rechromatography of the gum from fractions 30–85 over 800 g of silica gel (800-ml fractions) provided in fractions 1–30 (Bz to Bz–Chlf 10:3) 13.8 g of gummy mixture; continued elution (Bz–Chlf 10:3 to Bz–Chlf 1:1, fractions 31–77) gave solid. Recrystallization from ethyl acetate furnished 7.3 g of hymenolide (**8a**) which had mp 136–138°; $[\alpha]_D$ −48.6° ($CHCl_3$, *c* 1.4); ir bands at 3570, 3445, 1750, and 1660 cm^{-1}; nmr signals at 6.31 d (J = 2.5 Hz) and 5.60 (J = 2.0 Hz, exocyclic methylene conjugated with lactone), 5.11 d br, sharpens to dd (J_1 = 10.0, J_2 = 2.5 Hz) on addition of D_2O (H-3), 4.38 d (7.5, disappears on exchange, −OH), 4.31 (H-4), 4.1–3.3 c (nonequivalent CH_3CH_2O, confirmed by spin decoupling at 90 MHz), 1.25 t (J = 7.0 Hz, CH_3–CH_2O), 1.09 d (J = 7.0 Hz, C-10 methyl), and 1.05 ppm (C-5 methyl).

Anal. Calcd for $C_{17}H_{26}O_5$: C, 65.78; H, 8.44; O, 25.78. Found: C, 65.77; H, 8.61; O, 25.84.

Further elution gave 38.4 g of gummy mixture.

Anhydrohymenolide (10).—Acetylation of 126 mg of hymenolide with pyridine–acetic anhydride afforded a gum (**8b**) which could not be induced to crystallize: ir bands at 1760 (double strength, lactone and acetate) and 1220 cm^{-1}; nmr signals at 6.26 d (J = 2.5 Hz) and 5.57 d (J = 2 Hz, exocyclic methylene), 6.08 dd (J_1 = 10, J_2 = 2 Hz, H-3), 4.77 m (H-8), 4.31 (H-4), 3.3–4.2 c (2 protons, $-OCH_2CH_3$), 2.10 (acetate), 1.28 t (J = 7.0 Hz, $-OCH_2CH_3$), 1.10 d (J = 5.3 Hz, C-10 methyl), and 1.07 ppm (C-5 methyl).

The above substance, wt 810 mg, was heated at 190–200° in a nitrogen atmosphere for 1.5 hr. The solid product **10**, yield 695 mg, was recrystallized from petroleum ether–ether and then melted at 82–83°: $[\alpha]_D$ −146.4° ($CHCl_3$, *c* 1.195); ir bands at 1762 (γ-lactone) and 1662 cm^{-1} (strong, two double bonds).

Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.84; H, 8.27; O, 21.89. Found: C, 69.83; H, 8.23; O, 22.07.

Hydrogenation of Anhydrohymenolide.—A solution of 110 mg of **10** was hydrogenated with 60 mg of PtO_2 at room temperature for 6 hr. Filtration followed by evaporation *in vacuo* gave a gum which was chromatographed over 12 g of silica gel (40-ml fractions). Fractions 1–14 (petroleum ether–Bz 1:1 and Bz) yielded a trace of gum. Fractions 15–25 (Bz–Chlf 5:1 to 2:1) gave 50 mg of solid material (single spot on tlc) which after recrystallization from petroleum ether melted at 73–75° and was identical in all respects with **15b** from hydrolysis of hymenoxynin with ethanolic HCl. Further elution with Bz–Chlf 2:1 and 1:1 gave 42 mg of solid **16** (single spot on tlc) which was recrystallized from ethyl acetate–petroleum ether and had mp 154.5–156°; $[\alpha]_D$ −121.2° ($CHCl_3$, *c* 0.85); ir bands at 1738 and 1661 cm^{-1}; nmr signals at 5.00 m (H-8), 4.28 (H-4), 3.67 c (4 protons, H-3 and CH_3CH_2O–), 1.85 br (C-11 methyl), 1.24 t (J = 7 Hz, CH_3CH_2O–), 0.93 (C-5 methyl), and 0.90 d (J = 6 Hz, C-10 methyl).

Anal. Calcd for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90; O, 21.74. Found: C, 69.30; H, 8.72; O, 21.63.

Dehydrochymenolide (9).—A solution of 263 mg of hymenolide in 1.5 ml of pyridine was added to 245 mg of CrO_3 in 1 ml of pyridine, set aside at room temperature overnight, diluted with water, and extracted with ethyl acetate. The organic layer after washing and drying furnished 234 mg of solid material which was recrystallized from ethyl acetate and had mp 178–181°; $[\alpha]_D$ −56.2° (*c* 0.925, $CHCl_3$); ir bands at 1756 and 1661 cm^{-1}; nmr signals at 6.32 d (J = 2.5 Hz) and 5.60 d (J = 2.0 Hz, exocyclic methylene group), 4.78 m (H-8), 4.78 (H-4), 3.4–4.2 m (2 protons, CH_3CH_2O–), 1.28 t (J = 7 Hz, CH_3CH_2O–), 1.10 d (J = 6 Hz, C-10 methyl), and 1.05 ppm (C-5 methyl).

Anal. Calcd for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84; O, 25.94. Found: C, 66.40; H, 7.88; O, 25.82.

Oxidation of 305 mg of hymenolide in 2 ml of acetone with 6 ml of Jones reagent at room temperature and work-up in the usual fashion gave, in the neutral fraction, 97 mg of **9**, mp 178–181°. The acid fraction afforded 83 mg of deethyldehydrohymenolide (**11**), mp 145–146° (*vide infra*).

Deethyldehydrohymenolide (11).—A solution of 87 mg of **8a** in 1.5 ml of acetone–water (4:1) and 0.5 ml of concentrated HCl was allowed to stand at room temperature for 5 hr, diluted with water, and extracted with ethyl acetate. Evaporation of the washed and dried extract afforded 80 mg of **11** which was recrystallized from ethyl acetate and melted at 145–146°: $[\alpha]_D$ −2.7° (*c* 0.74, $CHCl_3$); ir bands at 1753a and 1660 cm^{-1}; nmr signals at 6.33 d (J = 2.5 Hz) and 5.68 d (J = 2 Hz, exocyclic methylene), 5.23 (H-4), 4.81 m (H-8), 1.09 d (J = 7 Hz, C-10 methyl), and 1.03 ppm (C-5 methyl).

Anal. Calcd for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19; O, 28.54. Found: C, 63.93; H, 7.16; O, 28.84.

$NaBH_4$ Reduction of 11.—A solution of 370 mg of **11** in 3 ml of methanol was allowed to stand overnight with an excess of $NaBH_4$, diluted with water, acidified with dilute HCl solution, and extracted with ethyl acetate. The washed and dried extract was evaporated at reduced pressure and the residue recrystallized from acetone. The first crop consisted of 86 mg of themoidin (dihydrofloribundin, **5a**), mp 212–214°, which was identical in all respects with an authentic sample. The material in the mother liquors was recrystallized from ethyl acetate. This afforded 63 mg of floribundin, mp 142–143°, which was identical in all respects with an authentic sample.

Reactions of Paucin. (A).—A solution of 70 mg of paucin in 1 ml of absolute pyridine and 0.5 ml of acetic anhydride was allowed to stand at room temperature overnight, diluted with water, and extracted with ethyl acetate. The washed and dried extracts were evaporated and the residual solid, wt 98 mg, was recrystallized from methanol. Triacetylpaucin had mp 240–242° (lit. 241–243°); $[\alpha]_D$ +38.8° (pyridine, *c* 0.8); ir bands at 1755 (very strong); 1224 nmr signals at 6.37 (J = 2.5 Hz) and 5.78 (J = 2 Hz, exocyclic methylene), 5.5–3.8 c (9 protons), 2.12, 2.07, 2.03 (acetates), 1.20 d (J = 6.0 Hz, C-10 methyl), and 0.98 ppm (C-5 methyl).

(B).—A mixture of 0.3 g of paucin, 5 ml of acetic acid, and 5 ml of water was refluxed for 5 hr until all starting material had disappeared (tlc); the mixture was cooled, diluted with water, and extracted with ethyl acetate. The washed and dried extract was evaporated and the gummy residue, wt 223 mg, was chromatographed over 10 g of silica gel. Benzene (2 fractions, 40 ml each) eluted a trace of gum; fractions 3–5 (benzene–ethyl acetate, 10:1) eluted 110 mg of solid which was recrystallized from ethyl acetate, mp 160–162° (lit. mp 159–160°). Mixture melting point and ir and nmr spectrum identified this substance as aromatin (**12**).[27]

Tetraacetylhymenoxynin (14b).—Acetylation of 68 mg of hymenoxin with 1 ml of absolute pyridine and 0.5 ml of acetic anhydride at room temperature overnight followed by dilution with water and extraction with ethyl acetate yielded, after washing and drying of the organic extract and evaporation *in vacuo*, 113 mg of solid tetraacetate. The product was recrystallized from ethyl acetate and had mp 176–177°; $[\alpha]_D$ +15.6° ($CHCl_3$, *c* 0.96); nmr signals (100 MHz, $CDCl_3$), 5.13 c (3 superimposed protons, –CHOAc), 4.79 d (J = 7 Hz, H_2, α-anomeric proton) superimposed on 4.7 c (2 protons, H-8 and –CHOAc), 4.20 (H-4) superimposed on 4.3–4.0 c (2 protons), 3.95 and 3.86 m (2 protons, H-3), 2.08, 2.03, 2.03, 2.00 (4 acetates), 1.14 d (J = 7 Hz), and 1.07 d (J = 7 Hz, C-10 and C-11 methyls), 1.00 ppm (C-5 methyl).

Hydrolysis of Hymenoxynin. (A).—A mixture of 200 mg of hymenoxynin, 2 ml of methanol, and 1 ml of concentrated HCl was allowed to stand at room temperature overnight, diluted with water, and extracted with ethyl acetate. Evaporation of the washed and dried extract furnished 110 mg of **15a** which was recrystallized from ether–petroleum ether and had mp 113–114°; $[\alpha]_D$ −83.5° ($CHCl_3$, *c* 0.635); ir bands 2828 (methoxyl), 3.70 and 2.93 m (H-3), 1.14 d (J = 6 Hz) and 1.10 (J = 5.5 Hz, C-10 and C-11 methyl), 1.07 ppm (C-5 methyl).

Anal. Calcd for $C_{16}H_{26}O_4$: C, 68.55; H, 8.63; O, 22.83. Found: C, 68.43; H, 8.95; O, 23.17.

The aqueous layer from the hydrolysis was concentrated to 20 ml at reduced pressure, neutralized with 0.2 g of $CaCO_3$, filtered, and evaporated at reduced pressure. The residue, wt 0.18 g, was mixed with 0.2 g of phenylhydrazine hydrochloride, 0.3 g of sodium acetate, and 2 ml of water, heated on the water bath for 10 min, cooled, and filtered. The yellow product was recrystallized from methanol and identified as glucosazone, mp 210–211°, by comparison with an authentic sample prepared from D-(+)-glucose in the usual manner.

Hydrolysis of 115 mg of the acetal **15a** with 1 ml of acetone–water (4:1) and 0.5 ml of concentrated HCl at room temperature for 2 days, dilution with water, extraction with ethyl acetate, and processing of the organic extract in the usual way resulted in 120 mg of a crude lactol **15c**. Recrystallization from acetone furnished 86 mg of which had mp 318–320° dec; $[\alpha]_D$ −118.4° ($CHCl_3$, *c* 0.38); ir bands at 3490, and 1762 cm^{-1}; nmr signals at 4.85 m (H-8), 4.51 (H-4), 3.75 and 2.95 m (H-3), 1.16 (C-5 methyl), 1.14 d and 1.11 d (J = 6.0 Hz, C-10 and C-11 methyls).

(B).—Hydrolysis of 128 mg of hymenoxynin in the manner described in the previous section using ethanolic HCl and work-up in the usual fashion furnished, after recrystallization from petroleum ether, the ethoxy derivative **15b** which had mp 73–75°; $[\alpha]_D$ −94.1° ($CHCl_3$, c 0.85); nmr signals at 4.77 m (H-8), 4.12 (H-4), 3.35–4 c (3 protons, H-3a and CH_3CH_2O–), 3.0 m (H-3_b), 1.22 t (J = 7 Hz, CH_3CH_2O–), 1.15 (J = 5 Hz,) and 1.08 d (J = 5.5 Hz, C-10 and C-11 methyl), and 1.06 ppm (C-5 methyl).

This substance was identical in all respects with the less polar material obtained by hydrogenation of anhydrohymenolide (10).

Anal. Calcd for $C_{17}H_{28}O_4$: C, 68.69; H, 9.52; O, 21.59. Found: C, 68.80; H, 9.45; O, 21.66.

Registry No.—**2**, 16983-23-6; **3**, 25062-22-0; **4a**, 25062-24-2; **4b**, 25062-25-3; **5a**, 25062-26-4; **5b**, 25062-27-5; **6**, 25080-56-2; **7**, 25062-28-6; **8a**, 25062-29-7; **8b**, 25062-30-0; **9**, 25062-31-1; **10**, 25062-32-2; **11**, 25062-33-3; **14a**, 25062-34-4; **14b**, 25062-35-5; **15a**, 25062-36-6; **15b**, 25062-37-7; **15c**, 25062-38-8; **16**, 25080-57-3; **17**, 25062-23-1; **18**, 25062-40-2.

Conversion of Solasodine to Solafloridine and Dihydrosolacongestidine Acetate

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The two major steroidal alkaloids, solafloridine and solacongestidine, isolable from *Solanum congestiflorum* have been synthesized from solasodine. In the conversion acetylsolasodine is reduced to 3-acetyltetrahydrosolasodine, converted to the N-carbobenzoxy derivative, and oxidized to the 3-acetyl-16-oxo-N-carbobenzoxy compound. Sodium–propanol reduction affords the desired 16α epimer, dihydrosolafloridine, convertible to solafloridine by dehydrohalogenation of the N-chloro derivative. Thioketalization of the 3-acetyl-16-oxo-N-carbobenzoxy compound followed by Raney nickel reduction yields dihydrosolacongestidine acetate.

In a recent publication,[2] the isolation and structure proof of the steroidal alkaloids, solacongestidine (I) and solafloridine (II), from *Solanum congestiflorum* were reported. Owing to the time-consuming, laborious procedure involved in isolation and poor yield of the alkaloids from the plant, an alternate source was sought for these compounds when a demand for more alkamine, particularly solafloridine (II), arose for other projects.

Starting from solasodine[3] (III), a readily available steroidal alkaloid having the correct stereochemical configuration, the conversion was achieved in the following manner. O-acetylsolasodine[4] (IV) prepared from the reaction of solasodine (III) with acetic acid containing *p*-toluenesulfonic acid was reduced with sodium borohydride to O-acetyldihydrosolasodine (V) and in turn reduced catalytically (Pd–C) to O-acetyltetrahydrosolasodine[5] (VI). Conversion of VI to the N-carbobenzoxy-3-acetyl derivative (VII) with carbobenzoxy chloride and oxidation with Kiliani's reagent[6] in acetone to the 16-oxo compound (VIII) followed by reduction with sodium–2-propanol afforded the 16α-hydroxyl bearing dihydrosolafloridine[2] (IX) in good yields. A somewhat lesser yield was obtained by reduction with lithium–ammonia. This was accounted for by the recovery of considerable deacetylated starting material, VIIIa.[7] Compound IX, thus prepared, agreed in properties (melting point, mixture melting point, ir) with that obtained from the reduction of the natural product. The pathway outlined above, we believe, is an improvement over the published partial synthetic procedure[8] since the stereochemistry at C-20 and C-25 is unaffected throughout these reactions. The yield of the N-carbobenzoxy compound (VII) is diminished somewhat by the formation of a by-product assigned the structure XII either formed by the interaction of VI with some phosgene liberated during the reaction or ring closure of the debenzyloxy product of VII with the C_{16}–OH function.[9] The structure of XII was confirmed by its synthesis from the reaction of VI with phosgene. It should be noted in passing that the sodium borohydride reduction of the 16-oxo compound VIII afforded only the 16β-hydroxy isomer, VII, as expected.

Finally dihydrosolafloridine (IX) was converted to solafloridine (II) by dehydrohalogenation of the N-chloro compound in the manner reported by Schreiber and Adam.[8]

The N-carbobenzoxy-16-oxo compound (VIII) served as a convenient starting point for the preparation of dihydrosolacongestidine acetate (XI). This was accomplished by thioketalization of VIII with ethanedithiol which yielded the crystalline thioketal X. Desulfurization of the thioketal moiety with Raney nickel led to concomitant elimination of the N-carbobenzoxy function to afford the desired dihydrosolacongestidine acetate[2] (XI). Compound XI exhibited properties (melting point, mixture melting point, ir, mass spectrum) indistinguishable from those derived from solacongestidine. The compound like dihydrosolafloridine

(1) Visiting Scientists: G. Kusano (1969–present) and N. Aimi (1968–1969).

(2) Y. Sato, H. Kaneko, E. Bianchi, and H. Kataoka, *J. Org. Chem.*, **34**, 1577 (1969).

(3) For a general review, see K. Schreiber in "The Alkaloids," Vol. X, R. H. F. Manske, Ed., Academic Press, New York, N. Y., 1968, Chapter 1.

(4) We are indebted to Dr. J. A. Beisler of this laboratory for working out this procedure. H. Rochelmeyer, *Arch. Pharm.* (Weinheim), **277**, 329 (1939), report mp 193–194°.

(5) Compound VI can also be prepared in somewhat reduced yields by the direct catalytic reduction (PtO_2–HAc) of O-acetylsolasodine (IV).

(6) H. Kiliani, *Ber.*, **46**, 676 (1913). A solution of 53 g of chromium trioxide and 80 g of concentrated sulfuric acid in 400 g of water was used.

(7) In two runs the yield of the deacetylated starting material, VIIIa, was approximately the same. Perhaps the insolubility of the compound prevents its further participation in the reaction. We intend to study this reaction further.

(8) K. Schreiber and G. Adam, *Justus Liebigs Ann. Chem.*, **666**, 176 (1963).

(9) It was suggested by one of the referees that formation of XII could have resulted from the attack of 16β-OH on the carbonyl function of the carbobenzoxy group of VII followed by loss of the benzyloxy ion. However, we had observed that in the repeat runs, vigorous agitation or stirring of the reaction flask resulted in negligible yields of XII. Hence, it was thought that in the earlier runs, due to insufficient agitation of the immiscible phase, neutralization was incomplete, and the resulting local acidic conditions led to prior debenzyloxylation.