Structure Elucidation of Sesquiterpene Dilactones from *Mikania Scandens* (L.) Willd.[1]

WERNER HERZ, P. S. SUBRAMANIAM, P. S. SANTHANAM, K. AOTA, AND ALLEN L. HALL

Department of Chemistry, Florida State University, Tallahassee, Florida 32306

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Six new sesquiterpene dilactones—mikanolide, dihydromikanolide, scandenolide, dihydroscandenolide, deoxymikanolide, and miscandenin—have been isolated from above-ground parts of *Mikania scandens* (L.) Willd. The first five are germacranediolides whose structure and stereochemistry were determined by a combination of chemical methods and nmr techniques and by correlation of deoxymikanolide with the germacranediolide isabelin. Miscandenin is an elemanediolide containing a dihydrooxepine ring, whose formation can be rationalized as involving the Cope rearrangement of a 1,10-deoxymikanolide precursor.

Extracts of many members of the large genus *Mikania* (family *Compositae*, tribe *Eupatorieae* Cass., subtribe *Ageratinae* Less.) which is abundantly represented in the western hemisphere, are used as folk medicine within their respective ranges. Since we suspected, on phylogenetic grounds, the presence of sesquiterpene lactones, we undertook an examination of accessible *Mikania* species. In the present paper we report the isolation and structure determination of six lactones from *Mikania scandens* (L.) Willd. (climbing hempvine).[2,3] Five of these belong to the class of novel sesquiterpenoid dilactones of the germacrane type some of which are reported to exhibit tumor-inhibitory activity.[3,9] The sixth represents an interesting and hitherto undescribed variant resulting from a germacradiene–elemadiene interconversion.

Table I lists these dilactones in the order of their elution. Isolation was effected by a combination of chromatography over silicic acid and fractional crystallization. Relative yields of mikanolide and dihydromikanolide, which invariably were the major constituents, varied depending on the date and location of collection. Scandenolide was next in abundance while the remaining three were present in minor amounts only. In fact, miscandenin and desoxymikanolide appeared to be absent from some collections (see Experimental Section).

TABLE I

CONSTITUENTS OF *Mikania scandens* (L.) WILLD.

Compd	Molecular formula	Mp, °C	$[\alpha]_D$, degree
Miscandenin	$C_{15}H_{14}O_5$	232–235	−181.4
Mikanolide	$C_{15}H_{14}O_6$	226–228	+53.4
Dihydromikanolide	$C_{15}H_{16}O_6$	241–244	+91.1
Desoxymikanolide	$C_{15}H_{16}O_5$	198–200	+98.9
Scandenolide	$C_{17}H_{18}O_7$	230–234	+62.0
Dihydroscandenolide	$C_{17}H_{20}O_7$	278–280	+83.3

Mikanolide and Dihydromikanolide.—The presence of partial structure A, encountered in many sesquiterpene lactones of *Compositae*, in mikanolide (**1**) was suggested by the uv [λ_{max} 206 nm (ϵ 16700)] and ir spectrum (bands at 1767, 1752, and 1661 cm^{-1}). This was established by ozonolysis which liberated formaldehyde and by the nmr spectrum (Table II) which exhibited the diagnostic[10] H_a and H_b doublets at 6.20 and 5.92 (J = 3.5 Hz) and a complex multiplet (H_d) at 4.72 ppm.[11] Double-resonance experiments at 90 and 100 MHz (Table III and Experimental Section) involving H_a, H_b, H_c, and H_d confirmed the presence of A.

Partial hydrogenation of **1** (Pd–$CaCO_3$) resulted in the formation of a dihydro derivative **2**, ir bands at 1760 (double intensity) and 1650 cm^{-1}, which was identical with dihydromikanolide isolated from the plant. In the nmr spectrum of **2**, the H_a and H_b doublets of **1** were replaced by a methyl doublet at 1.28 ppm, reflecting the change brought about by reduction of A. Total reduction of **1** led to tetrahydromikanolide (**3**) which had ir bands at 1800 and 1755 cm^{-1} and no uv absorption.

The presence of a second α,β-unsaturated lactone group in **1** was surmised from the ir spectra of **1**, **2**, and **3** which exhibited two strong bands in the γ-lactone region and the uv spectrum of **2** which, despite saturation of chromophore A, displayed strong absorption at 217 nm (ϵ 8800).[12]

That partial structure B represented this second chromophore was indicated by the nmr spectra of **1** and **2** which displayed a narrow doublet at 7.56 (J = 1.7 Hz, H_e, 7.43 in **2**) absent in **3** and a narrowly split multiplet at 5.42 (H_f, 5.28 in **2**) which in **3** had moved upfield to

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

(2) Part of this material has been published in preliminary form: W. Herz, P. S. Santhanam, P. S. Subramaniam, and J. J. Schmid, *Tetrahedron Lett.*, 3111 (1967).

(3) The *Mikania scandens* aggregate of North American and pantropical distribution has been treated by Robinson.[4] The work described in the present paper deals with the constituents of *M. scandens* (L.) Willd. *sensu strictiori*, found in wet thickets and swamps, chiefly near the coast, of the southeastern United States. Since our original publication,[2] mikanolide and dihydromikanolide have also been isolated[5] from *M. cordata* (Burm. f.) Robinson, an Afro-Malaysian member of the complex and from *M. batatifolia* DC,[6] a segregate found in Cuba and the Florida Keys. Work on other *Mikania* species is in progress. Mikanolide has also been isolated from *Gaillardia fastigiata* Greene (tribe *Helenieae* Benth. and Hook.).[7]

(4) B. L. Robinson, *Contrib. Gray Herbarium Harv. Univ.*, **104**, 55 (1934).

(5) A. K. Kiang, K. Y. Sim, and S. W. Yoong, *Phytochem.*, **7**, 1035 (1968).

(6) W. Herz, P. S. Santhanam, H. Wagner, R. Höer, L. Hörhammer, and L. Farkas, *Tetrahedron Lett.*, 3419 (1969).

(7) W. Herz, S. Rajappa, S. K. Roy, J. J. Schmid, and R. N. Mirrington, *Tetrahedron*, **22**, 1907 (1966).

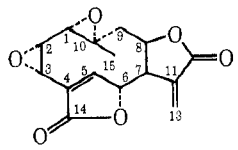
(8) S. M. Kupchan, Y. Aynehchi, M. Cassady, A. T. McPhail, G. A. Sim, H. K. Schnoes, and A. L. Burlingame, *J. Amer. Chem. Soc.*, **88**, 3674 (1966); S. M. Kupchan, Y. Aynehchi, J. M. Cassady, H. K. Schnoes, and A. L. Burlingame, *J. Org. Chem.*, **34**, 3867 (1969).

(9) H. Yoshioka, T. J. Mabry, and H. E. Miller, *Chem. Commun.*, 1679 (1968); H. Yoshioka and T. J. Mabry, *Tetrahedron* **25**, 4767 (1967).

(10) W. Herz, H. Watanabe, M. Miyazaki, and Y. Kishida, *J. Amer. Chem Soc.*, **84**, 2601 (1962).

(11) Measured in DMSO-d_6, unless otherwise specified, on a Varian A-60 nmr spectrometer.

(12) *Cf.* the uv maxima of dihydroelephantolide [λ_{max} 211 nm (ϵ 8700)] and dihydroelephantol [λ_{max} 211 nm (ϵ 9600)]. Subtraction of the uv spectrum of **2** from the uv spectrum of **1** gave a difference curve with λ_{max} 205 nm (ϵ 8800) and 225 (4400).



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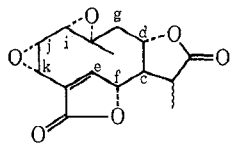

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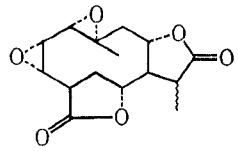

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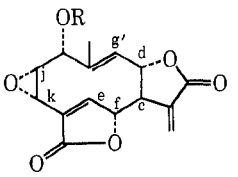

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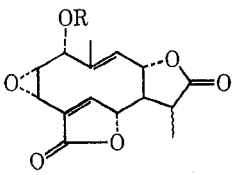

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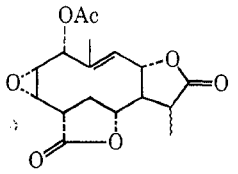

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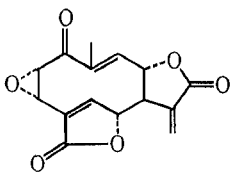

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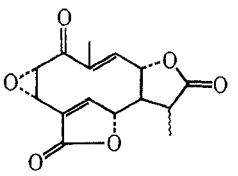

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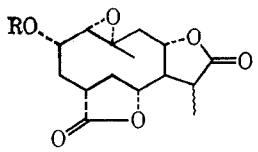

9a, R = Ac
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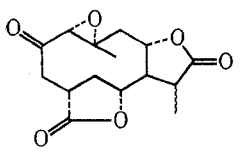

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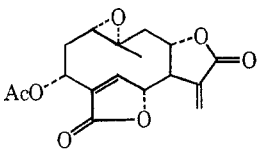

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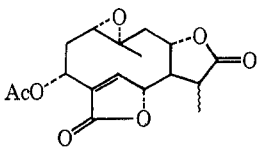

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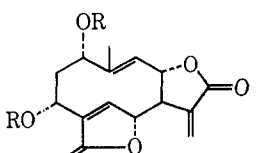

14a, R = H
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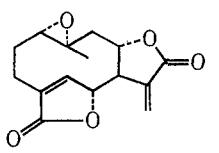

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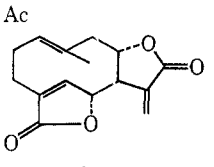

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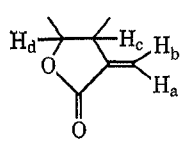
4.68 ppm and merged with the signal of H_d.[13] This was again confirmed by double-resonance experiments (*vide infra*).

(13) *Cf.* the corresponding resonances in the nmr spectra of elephantopin and its derivatives,[8] ovatodiolide,[14] linderalactone, and neolinderane.[15]

(14) H. Immer, J. Polonsky, R. Toubiana, and H. D. An, *Tetrahedron*, **21**, 2117 (1965).



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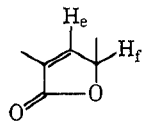


B

The evidence presented so far accounted for four of the six oxygen atoms present in **1**. The absence of hydroxyl groups revealed by the ir and nmr spectra and the absence of ketone groups indicated by negative chemical tests and the CD curve (no Cotton effect in the 290-nm region) suggested that the remaining two oxygen atoms might be ethereal. This conclusion was strongly reinforced by the presence in the nmr spectrum of **1** of a one-proton signal at 3.96 (broadened doublet, J_1 = 3.5, J_2 = 1.1 Hz) and a complex two-proton signal at 3.36 ppm.[16] On this basis **1**, because of the presence of only one quaternary methyl group (singlet at 1.01 ppm),[17] had to possess a single ten-membered carbocyclic ring.

The presence of three protons assignable to carbon atoms bearing two ethereal oxygens prompted us to postulate the carbon atom carrying the quaternary methyl group as the fourth point of attachment of the two ether bridges, as indicated in partial structure C. Confirmative evidence came from the following transformation of mikanolide. Treatment of **1** with excess acetic anhydride in the presence of *p*-toluenesulfonic acid under reflux gave in moderate yield an olefinic acetate **4a**. In the nmr spectrum of this substance, the quaternary methyl of **1** was replaced by a vinyl methyl at 1.72 (J = 0.7 Hz), the methylene multiplet of **1** (H_{g_1} and H_{g_2} of C) was replaced by a new vinyl proton at 5.32 (broad doublet, $H_{g'}$ of D, A part of AB system with weak allylic coupling to H_h and strong coupling—10.2 Hz—to H_d), H_d had experienced a slight downfield shift and simplification to a triplet at 4.82 (B of AB system, J = 10.2 Hz), and H_i, formerly in the 3.36 cluster of **1**, had moved downfield to 5.52 ppm (doublet, J = 4.7 Hz). These spectral changes were interpretable on the basis of the transformation C to D.[18]

In agreement with partial structure D, acid hydrolysis of **4a** afforded the allylic alcohol **4b** which had the requisite spectral properties (Table II and Experimental Section).[20] In fact mikanolide itself was converted in good yield into **4b** on treatment with methanol-hydrochloric acid. Compounds **5a**, **5b**, and **6** with the expected properties were similarly formed from **2** and

(15) B. S. Joshi, V. N. Khamat, and T. R. Govindachari, *ibid.*, **23**, 261, 267 (1967).

(16) Integration in this region was deceptive in DMSO-d_6 solution because of the superposition of the water signal. The nmr spectrum in pyridine-d_5 indicated the presence of three protons near 3.4 ppm. That one of these had to be assigned to H_6 was established by spin decoupling. The remaining two protons and the proton responsible for the signal at 3.96 ppm were therefore identified with hydrogen on carbon carrying ether oxygen.

(17) This methyl group was apparently shielded in **1** and **2** by the double bond of partial structure B because it displayed the customary chemical shift of CH_3CO at 1.35 ppm in the nmr spectrum of **3**. The only other resonance, not yet mentioned, in the nmr spectrum of **1** was a complex two-proton multiplet in the methylene region (2.03 ppm).

(18) It is of interest that the reagent combination used in this experiment did not effect cyclization to a eudesmane derivative as observed, for example, in the pyrethrosin series.[19]

(19) D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 150 (1957); D. H. R. Barton, O. C. Böckman, and P. de Mayo, *ibid.*, 2263 (1960); S. Iriuchijima and S. Tamura, *Tetrahedron Lett.*, 1965 (1967).

(20) That no rearrangement had taken place under these conditions was shown by reacetylation of **4b** to **4a**.

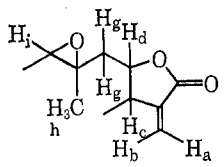
TABLE II

NMR SPECTRA OF CONSTITUENTS OF *Mikania scandens* AND DERIVATIVES[a]

Compd	H-1	H-2	H-3	H-5	H-6	H-8	H-9	H-13	C-10 Me	C-11 Me	Misc
1	3.36 (c)	3.36 (c)	3.96 (dbr, 3.5)	7.56 (d, 1.7)	5.42 (nm)	4.72 (c)	2.03 (c)	6.20 (d, 3.5) 5.92 (d, 3.5)	1.01		
2	3.30 (c)	3.30 (c)	3.95 (dbr, 3.5)	7.52 (d, 2.0)	5.36 (nm)	4.56 (c)	2.0 (c)		0.98	1.28 (d, 6.5)	
3	3.9 (c)	3.9 (c)	3.9 (c)		4.68 (c)	4.68 (c)			1.35	1.17 (d, 6.5)	
4a	5.22 (d, 4.7)	3.62 (t, 4.7)	3.90 (nm)	7.72 (t, 1.5)	5.78 (nm)	4.88 (t, 10.2)	5.32 (dbr, 10.2)	6.15 (d, 3.2) 5.95 (d, 3.2)	1.72 (d, 0.7)		2.10[b]
4b	4.29 (br)[c]	3.42 (t, 4.7)	3.75 (nm)	7.50 (t, 1.5)	5.64 (nm)	4.83 (t, 10.2)	5.14 (dbr 10.2)	6.09 (d, 3.2) 5.95 (d, 3.2)	1.70 (d, 0.7)		5.52 (d, 3.5)[d]
5a	5.30 (d, 4.7)	3.67 (t, 4.7)	3.87 (nm)	7.78 (t, 1.5)	5.50 (nm)	4.87 (t, 10.2)	5.12 (dbr, 10.2)		1.73 (d, 0.7)	1.24 (d, 6.5)	2.13[b]
5b	4.30 (br)[b]	3.41 (t, 4.7)	3.87 (nm)	7.78 (t, 1.5)	5.50 (nm)	4.87 (t, 10.2)	5.12 (dbr, 10.2)		1.73 (d, 0.7)	1.23 (d, 6.5)	5.75 (br)[d]
7		4.40 (d, 4.7)	4.16 (nm)	7.37 (t, 1.5)	5.81 (nm)	4.71 (t, 10.2)	5.61 (dbr, 10.2)	6.31 (d, 3.2) 6.01 (d, 3.2)	2.08 (d, 1.1)		
8		4.48 (d, 4.7)	4.25 (nm)	7.58 (t, 1.5)	5.50 (nm)	4.62 (t, 10.2)	5.56 (dbr, 10.2)		2.0 (d, 1.1)	1.22 (d, 6.5)	
9a	3.5 (d, 10)	4.72 (c)			4.72 (c)	4.72 (c)			1.33	1.15 (d, 6.5)	1.99[b]
9b	3.15 (d, 10)	3.5 (c)[e]			4.75 (c)	4.75 (c)			1.30	1.15 (d, 6.5)	4.92 (d)[d]
11[f]	4.26				4.8 (c)	4.8 (c)			1.15	1.20 (d, 6.5)	
12	2.95 (dd, 12.0, 2.5)		5.55 (m)	7.83 (br)	5.55 (m)	4.7 (m)		6.18 (d, 3) 6.00 (d, 3)	1.09		3.5,[g] 2.12[b]
13	3.0 (dd)		5.5 (m)	7.82 (t, 1)	5.5 (m)	4.5 (m)			1.50	1.25 (d, 7)	2.12[b]
14a	4.5 (c)	~2.6[h] ~2	4.5 (c)	7.42 (t, 1)	5.66 (m)	5.0 (m)	5.0 (m)	6.08 (d, 3) 5.89 (d, 3)	1.60 (br)		3.4 (m)[g] 5.43 (d, 4)[d] 4.74 (d, 4)[d]
14b	5.5 (c)	~2.6[h] ~2	5.3 (c)	7.8 (t, 1)	5.78 (m)	5.05 (t, 10)	5.50 (dbr, 10)	6.08 (d, 3) 5.90 (d, 3)	1.65 (br)		3.4 (m)[g] 2.12,[b] 1.98[b]
15	2.85 (dd, 12, 2.5)	1.8 (m)		7.70 (t, 1)	5.42 (br)	4.7 (m)		6.18 (d, 3) 5.99 (d, 3)	1.10		3.45 (m)[g]
17	4.84 (d, 8.5)	6.23 (d, 8.5)	7.24 (d, 3.5)	3.5 (dd, 7, 3.5)	4.98 (dd, 10, 7)	4.18 (td, 11, 3.5)	1.9 (c)	5.98 (d, 3.5) 5.65 (d, 3.5)	1.26		2.84 (c)[g]
18	1.9 (c)	4.2 (m)	7.42 (d, 3.5)	3.5 (dd, 10, 3.5)	4.82 (dd, 10, 7)	4.2 (t, 11, 3.5)	1.9 (c)		1.18	1.15 (d, 7)	2.75 (m)[i]

[a] Spectra were run in DMSO-d_6 solution on a Varian A-60 nmr spectrometer using tetramethylsilane as internal standard.[6] Superimposed signals were frequently separated in pyridine-d_5 solution; such spectra are given in the Experimental Section. Chemical shifts are quoted in parts per million, signals being denoted in the usual way: d, doublet; dbr, broadened doublet; t, triplet; q, quartet; nm, narrow multiplet; c, complex signal whose center is given. Singlets are unmarked. Figures in parentheses are line separations in hertz. H-9 in **1** and **2** integrated for two protons, methyl signals for three protons, other signals had one-proton intensities. [b] Acetate. [c] Sharpens to doublet on addition of D_2O. [d] Hydroxyl proton, disappears on addition of D_2O. [e] Narrows on addition of D_2O. [f] Narrows on addition of D_2O. [f] Run at 100°. [g] H-7. [h] Partially obscured by DMSO signal. [i] H-11.

3. The incorporation of partial structure A into C and D as illustrated was further demonstrated by spin-decoupling experiments involving H_a, H_b, H_c, H_d, H_g and $H_{g'}$, H_h, and H_i of **4a** detailed in Table III.



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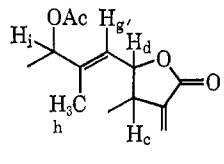

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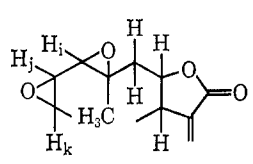


E

Inspection of the nmr spectra of **4a**, **4b**, **5a**, and **5b** suggested, and the data of Table III confirmed, that H_i was the X component of an ABX system where H_A (*e.g.*, in **4a**) was at 3.62 and H_B at 3.90 ($J_{AX} = J_{AB} = 4.7$ Hz, $J_{BX} = 0$ Hz) and where H_B in turn was weakly coupled to H_e and H_f of partial structure B ($J_{H_BH_e} = 1.5$, $J_{H_BH_f} = 1.1$ Hz). Hence partial struc-

TABLE III

DOUBLE IRRADIATION OF **4a**[a]

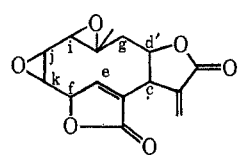
Signal irradd	Signal obsd	Change obsd	Inference
6.16 (H_a)	3.38 (m, H_c)	Sharpened	
5.95 (H_b)	3.38 (m, H_c)	Sharpened	
3.38 (m, H_c)	6.16 (d, H_a)	Collapsed to s	$J_{a,c} = 3.2$
	5.95 (d, H_b)	Collapsed to s	$J_{b,c} = 3.2$
	5.80 (nm, H_f)	Affected	
	4.92 (t, H_d)	Collapsed to d	$J_{c,d} = 10$
4.92 (H_d)	5.35 (dbr, $H_{g'}$)	Collapsed to br?[b]	
	3.38 3(m, H_c)	Simplified	
5.35 ($H_{g'}$)	4.92 (t, H_d)	Collapsed to d	$J_{d,g'} = 9.8$
	1.72 (d, H_h)	Collapsed to s	$J_{g',h} = 1$
5.27 (H_i)	3.63 (t, H_j)	Collapsed to d	$J_{i,j} = 5.1$
3.63 (H_j)	5.27 (dbr, H_i)	Collapsed to br[c]	$J_{i,j} = 5.1$
	3.91 (dt, H_k)	Collapsed to nm	
3.91 (H_k)	7.77 (t, H_e)	Collapsed to d	$J_{e,k} \sim 1$
	5.80 (nm, H_f)	Simplifies	
	3.63 (t, H_j)	Collapsed to d	$J_{j,k} = 4.2$
5.80 (H_f)	7.77 (t, H_e)	Collapsed to d	$J_{e,f} = 1.6$
	3.91 (dt, H_k)	Collapsed to dd	
7.77 (t, H_e)	3.91 (t, H_h)	Collapsed to dd	$J_{f,k} = 1.2$
	5.80 (nm, H_f)	Somewhat resolved[d]	

[a] Run on Bruker 90-MHz spectrometer in DMSO-d_6 solution. Limits of error 0.1 Hz. [b] Difficult to determine owing to proximity irradiation frequency to signal observed. [c] Broadening due to allylic coupling to $H_{g'}$ ($J < 0.5$ Hz). [d] Line broadening due to coupling to H_c ($J_{c,f} \sim 0.5$ Hz).

ture C could be expanded to E where $H_A = H_i$ and $H_B = H_k$.

In accordance with partial structures D and E, manganese dioxide oxidation of **4b** gave an α,β-unsaturated ketone **7** which had λ_{max} (after subtraction of the chromophore present in **1**) 250 and 310 nm (ϵ 3080 and 200)[21] and ir bands at 1778, 1768, 1700, 1675 (weak), and 1645 cm^{-1} characteristic of the two α,β-unsaturated lactones and a transoid conjugated ketone. In the nmr spectrum of **7** the signals of $H_{g'}$ and H_j had experienced the expected paramagnetic shift, now appearing at 5.61 and 4.40 ppm, respectively, and the resonance of H_j had collapsed to a doublet as required by E. The ketone **8**, λ_{max} (after subtraction of the chromophore of **2**) 255 and 318 nm (ϵ 1200 and 150) and ir bands at 1780, 1747, 1698, 1660, and 1635 cm^{-1}, with the expected nmr signals (Table II), was similarly formed from **5b**.

Partial formulas B and E together accounted for all atoms and functional groups of mikanolide and could be combined in two ways. The first of these possibilities, **1**, possesses the regular isoprenoid skeleton, but its adoption requires the assumption (see Table III) that, in the derivative **4a**, H-6 (H_f) and H-7 (H_c), although vicinal, are not coupled. The observed coupling between H_k and H_e would then be allylic and the coupling between H_k and H_f homoallylic. The second possibility, F, is biogenetically quite implausible but would explain the lack of coupling in **4a** between H_c and H_f. On the other hand it provides no simple rationale for the observed coupling between H_e and H_k.[23]



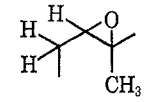
F

A clear decision in favor of **1** and against F was made possible by the observation that hydrogenation of mikanolide or dihydromikanolide to **3** was accompanied by formation of an alcohol **9b** obviously generated by hydrogenolysis of an allylic carbon–oxygen bond.[24,26]

Acetylation produced the acetate **9a**; spectral changes accompanying this transformation (Table II and Experimental Section) were consonant with the proposed formulas.[27] Furthermore, oxidation of **9b** with Jones reagent furnished a saturated ketone **11**, λ_{max} 285 nm (ϵ 68) and ir bands at 1780, 1770, and 1715 cm^{-1}, whose H-1 resonance had experienced the expected paramagnetic shift to 4.25 ppm and had collapsed to a singlet as required by the assigned structure. These transformations were not explicable in terms of F, but provided positive proof for formulation of mikanolide as **1** (devoid of stereochemistry).

Scandenolide and Dihydroscandenolide.—Ultraviolet [λ_{max} 209 nm (ϵ 15,250)], infrared (1770, 1747, and 1657 cm^{-1}), and nmr spectrum (Table II) indicated the presence of partial structures A and B in scandenolide (**12**). This was established in the now familiar manner by decoupling the resonances of H-13a and H-13b (H_a and H_b) at 6.18 and 6.00, H-7 (H_c) at 3.5, and H-8 (H_d) at 4.7 ppm on the one hand and H-5 (H_e) at 7.83 and H-6 (H_f) at 5.55 ppm on the other. That the relationship of scandenolide and dihydroscandenolide resembled that of **1** and **2** was evident from the spectra and was confirmed by partial hydrogenation of **12** to **13**. That **12** and **13** were acetates was suggested by the analysis and the ir spectrum which contained an additional band at 1739 cm^{-1} and was confirmed by the nmr spectrum which exhibited an acetate singlet at 2.12 ppm.

The nmr spectrum of scandenolide lacked the signals of H_j and H_k in partial structure E of mikanolide. Instead of the three-proton multiplet near 3.4 ppm (H_c, H_j, and H_k) there was the typical one-proton multiplet of H_c (H-7) at 3.5 ppm, a distinct one-proton doublet of doublets at 2.96 ppm attributable perhaps to epoxidic hydrogen in partial structure G, and a new signal at 5.55 ppm provisionally assigned to hydrogen under the acetate function. Because of the presence of high field multiplets which corresponded to two methylene or methinyl protons, it was logical to assign



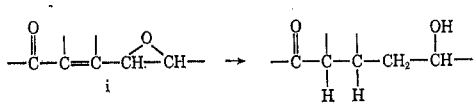
G

(21) Compare with the uv spectra of similarly constituted heliangine derivatives.[22] The low intensity may be attributed to steric deformation of the chromophore.

(22) S. Iriuchijima, S. Kuyama, N. Takahashi, and S. Tamura, *Agr. Biol. Chem.* (Tokyo), **30**, 511, 1152 (1966); H. Morimoto, Y. Sanno, and H. Oshio, *Tetrahedron*, **22**, 3173 (1966); M. Nishikawa, K. Kamiya, A. Takabatake, and H. Oshio, *ibid.*, **22**, 3601 (1966).

(23) Spin-decoupling experiments on mikanolide at 100 MHz, described in the Experimental Section, permitted clarification of all coupling constants and demonstrated the existence of vicinal coupling (J = 4.2 Hz) between H-6 and H-7 in mikanolide. However, at this stage this information could not be used to distinguish between formulas **1** and F because the superposition of the H-1, H-2, and H-7 resonances at 100 MHz did not allow us to assign the 4.2-Hz coupling specifically to the influence of H-1, H-2, or H-7.

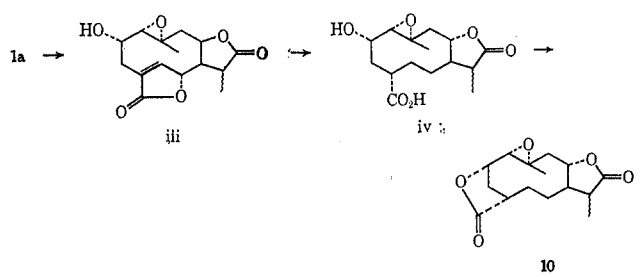
(24) Because of this the hydrogen uptake was always more than 2 molar equiv. Compare with the hydrogenolysis of magnamycin[25] in which i is converted into ii.



i ii

(25) R. B. Woodward, *Festschr. Arthur Stoll*, 524 (1957); *Angew. Chem.*, **69**, 50 (1957).

(26) A third minor product formed during the hydrogenation of **1** or **2** had infrared bands at 1770 and 1760 cm^{-1} and an R_f value very similar to that of **3**. The analytical data and nmr signals (see Experimental Section) indicated that it possessed structure **10** which could arise from further hydrogenolysis of an intermediate iii on the route to **9b** *via* iv. Relative yields of **3**, **9b**, and **10** in a typical run were *ca.* 5:1.5:0.5.



iii iv 10

(27) In the DMSO spectrum of **9b**, the signal of H-1 could not be discerned clearly as it was partially obscured by the DMSO–H_2O and other signals. However in pyridine solution it was clearly visible as a doublet at 3.7 ppm (10 Hz) and H-2 appeared at 4.29 ppm (shifted to 5.45 ppm—apparent octet—in the acetate **9a**).

to scandenolide formula **12** in which the 2,3-epoxide function of mikanolide was opened toward C-3. This was established in the following manner.

On treatment with 4% methanolic hydrochloric acid, scandenolide underwent a transformation parallelling the conversion of mikanolide into **4b**. The only additional feature in the product **14a** which requires mention at this stage was the simultaneous hydrolysis of the acetate function evident from the analysis and the nmr spectrum. Attempts at selective removal of one of the two hydroxyl groups, whose α protons were superimposed at 4.5 ppm, to effect a possible correlation with deoxymikanolide (*vide infra*) were not successful. However, the beautifully distinct 90-MHz nmr spectrum[28] of the diacetate **14b** permitted delineation and combination of all structural features (Table IV).

Irradiation at the frequencies of H-13a and H-13b identified the signal of H-7. Conversely, irradiation at the frequency of H-7 collapsed not only the signals of the exocyclic methylene group, but identified the neighboring protons H-6 and H-8. Which of the affected signals corresponded to H-8 became clear on irradiating at a frequency corresponding to the signal of H-9 (broadened doublet at 5.26 ppm). This collapsed the narrowly split (allylic coupling) C-10 methyl resonance and simplified the broadened triplet at 4.98 (H-8), but did not affect the narrowly split multiplet of H-6 at 5.46 ppm. H-6 was not only coupled vicinally to H-7 and H-5, but also (homoallylically, just as in **4a**) to a multiplet at 5.60 ppm which corresponded to hydrogen under the acetate group originally present in scandenolide. This hydrogen was coupled allylically, as in **4a**, to H-5 and vicinally to two geminally coupled protons of a methylene group whose signals were at 2.71 and 2.05 ppm. Each of these two high-field protons was in turn coupled to hydrogen under the acetate function introduced during the conversion of **12** into **14b** which must therefore be attached to C-1. This completed the structure proof of **14b** and therefore **12**.

Deoxymikanolide.—This minor lactone of *M. scandens* was intermediate in polarity between mikanolide and scandenolide. That it should possess structure **15** (devoid of stereochemistry) was apparent when its nmr spectrum (Table II) was compared with that of scandenolide. The spectrum was superimposed on that of **12** except for the absence of the signal of the acetate methyl and the proton under it. This was compensated for by a two-proton increase in the methylene region.

The appearance of a communication dealing with the structure of the germacranolide isabelin (**16**)[29] suggested the possibility of establishing a correlation of isabelin with deoxymikanolide. Indeed, peracid oxidation of isabelin[30] afforded in excellent yield a substance which was identical in all respects with deoxymikanolide, thus confirming its structure. Because isabelin has been related to cnicin of established relative and absolute configuration, the stereochemistry of deoxymikanolide at C-6, C-7, and C-8 was thereby settled. Furthermore inspection of an isabelin model indicated that reagent attack should occur preferentially from the α side. This would lead to the configuration at C-1 and C-10 depicted in **15**. In this orientation the C-10 methyl group is somewhat shielded by the 4,5 double bond as required by the nmr spectrum (Table III see also footnote 17). Additional evidence for this assignment will be cited in the sequel.

Stereochemistry of Mikanolide and Scandenolide.—A number of attempts were made to interrelate mikanolide and scandenolide with deoxymikanolide by removal of oxygen functions at C-2 and C-3. These were unsuccessful. Nevertheless, leaving aside biogenetic considerations, the pronounced similarity in chemical shifts and coupling constants evident from Table II and from the spin-decoupling experiments left practically no doubt that mikanolide, scandenolide, and deoxymikanolide possessed the same stereochemistry at C-1, C-6, C-7, C-8, and C-10. This was supported by the following observations.

Irradiation at the frequency corresponding to H-9 produced a 10% enhancement in the integrated intensity of the C-10 methyl signal of **4a** and an 11% enhancement in the integrated intensity of the C-10 methyl signal of **14b**.[31] The existence of an appreciable NOE showed that the newly introduced double bond was therefore *cis*, as indicated in the formulas. If **4b** and **14a** are formed from mikanolide and scandenolide by concerted reactions as seems likely because of the absence of $\Delta^{10,15}$ isomers, the required antiparallel orientation for the C-10 oxygen and C-9 hydrogen bonds which are broken in the elimination process leading to a *cis*-$\Delta^{9,10}$ olefin necessitates that mikanolide and scandenolide be formulated as 1,10-α-epoxides. As in the case of deoxymikanolide, the α configuration of the 1,10-epoxide should result in deshielding of the C-10 methyl group by the 4,5 double bond as was actually observed (Table II).[17]

An additional consequence of the α configuration of the 1,10-oxirane ring and a lactone stereochemistry corresponding to that of deoxymikanolide is the pronounced proximity of H-1, H-5, and H-8 apparent from the models of mikanolide (Figure 1) and **4a** (Figure 2) which should be reflected in relatively strong NOE's. Indeed, irradiation at the frequency of H-5 produced, for **4a**, a 26% enhancement in the in-

(28) Measured on a Bruker 90-MHz nmr spectrometer purchased with the aid of a grant from the National Science Foundation for which we express our thanks.

(29) In ref 9, the planar formulas of isabelin which is a mixture of two conformers and those of its derivatives are drawn so as to show a *cis* relationship between H-1 and the C-10 methyl group. The spatial formulas are drawn so as to show a *trans* relationship between H-1 and C-10 methyl. The latter appears to be correct. In our hands, irradiation[28] at either of the frequencies of H-1 collapsed the signals of the vinyl methyl group corresponding to the appropriate conformer, but produced no enhancement whatsoever in the integrated intensity of the C-10 methyl resonance.

The absence of a nuclear Overhauser effect indicates that H-1 and the C-10 methyl group of isabelin are *trans*, as shown in formula **16**. On the other hand irradiation at the two frequencies corresponding to H-8 of isabelin produced a 16% enhancement in the integrated intensity of the H-5 signals, as required by the formula which brings H-5 and H-8 into close proximity. Neither of the C-10 methyl signals was a simple doublet as reported.[9] The upfield signal appeared as a triplet, possibly due to the presence of homoallylic coupling ($J_{1,15} = J_{2a(?),15} = 0.65$ Hz), the lower methyl as a broadened doublet ($J_{1,15} = 1.2$, $J_{2a(?),15} < 0.5$ Hz).

(30) We are grateful to Dr. H. Yoshioka and Professor T. J. Mabry for a generous sample of this compound.

(31) An 11% increase in the intensity of the 1-acetate methyl signal of **14b** was also noted, but there was no effect on the intensity of the acetate methyl of **4a**. This is probably due to the greater conformational rigidity imposed on **4a** by the presence of the epoxide ring which results in somewhat different conformations for **4a** and **14a** also evident from the difference in the homoallylic coupling constants $J_{3,6}$ and the difference in the NOE's involving H-5 and H-8 (*vide infra*). This appears to produce preferred orientations for the acetate methyl closer to H-9 in **14b** than in **4a**.

TABLE IV

DOUBLE IRRADIATION OF **14b**[a]

Signal irradd	Signal obsd	Change obsd	Inference[b]
1.79 (C-10 Me)	5.26 (dbr, H-9)	Sharpens to d (10.6)	$J_{8,9}$ = 10.6 $J_{9,15}$ = 1.0
5.26 (H-9)	1.79 (d, C-10 Me)	Collapsed to s	
	4.98 (tbr, H-8)	Perturbed	
4.98 (H-8)	4.26 (dbr, H-9)	Collapsed to br	$J_{8,9}$ = 10.6
	3.28 (dtd, H-7)	Collapsed to td (3.5, 1.5)	
3.28 (H-7)	6.34 (dd, H-13a)	Collapsed to d (0.6)	$J_{7,13a}$ = 3.6
	5.70 (dd, H-13b)	Collapsed to d (0.6)	$J_{7,13b}$ = 3.2
	5.46 (ddd, H-6)	Collapsed to dd	
	4.98 (tbr, H-8)	Collapsed to dbr	
6.34 (H-13a)	5.70 (dd, H-13b)	Collapsed to d (3.2)	$J_{13a,13b}$ = 0.6
	3.28 (dtd, H-7)	Collapsed to ddd (9.6, 3.2, 1.2)	
5.70 (H-13b)	6.34 (H-13a)	Collapsed to d (3.6)	
	3.28 (dtd, H-7)	Collapsed to ddd (9.6, 3.6, 1.2)	
5.46 (H-6)	7.36 (dd, H-5)	Collapsed to d (1.6)	$J_{5,6}$ = 1.6 (allylic)
	5.75 (m, H-3)	Affected	
	3.28 (dtd, H-7)	Collapsed to dt (1.2, 3.5)	$J_{7,8}$ = 9.6
7.36 (H-5)	5.46 (ddd, H-6)	Collapsed to dd (2.7, 1.2)	$J_{6,7}$ = 1.2 $J_{5,6}$ = 2.7 H-3 signal is at 5.75
5.75 (H-3)	7.36 (dd, H-5)	Collapsed to d (1.4)	$J_{5,6}$ = 1.4
	5.46 (ddd, H-6)	Affected	
	2.71 (ddd, H-2a)	Collapsed to dd (15.5, 9.6)	$J_{2a,3}$ = 3.5[d]
	2.06 (ddbr, H-2b)[c]	Collapsed to dbr (15.5)	$J_{2b,3}$ = 3.1
2.71 (H-2a)	5.75 (m, H-3)	Affected	$J_{2a,2b}$ = 15.5[d]
	5.60 (dbr, H-1)	Collapsed to br[e]	$J_{1,2a}$ = 9.6[d]
5.60 (H-1)	2.71 (ddd, H-2a)	Collapsed to dd (15.5, 3.5)	$J_{1,2b}$ < 1.0
	2.05 (ddbr, H-2b)[c]	Affected	

[a] Run on Bruker 90-MHz nmr spectrometer in $CDCl_3$. [b] Limit of error 0.1 Hz. [c] Partially hidden under acetate signal. INDOR experiment using H-2b to monitor the sweep showed H-2b to be ddbr. [d] Values taken directly from a 4-Hz/cm scan without confirmation by spin decoupling. [e] Broadening due to $J_{1,9} \sim 0.5$ Hz and to a small coupling with H-8.

tegrated intensity of H-8, a 13% increase in the intensity of H-1,[32] and a 19% increase in the intensity of the vicinal H-6. Hence H-1, H-6, and H-8 were *cis* to each other and β.

The α orientation of the 2,3-oxirane ring of mikanolide and of the 3-acetoxy function of scandenolide postulated in the formulas is based on the magnitude of the observed coupling constants and on the impossibility of constructing a Dreiding model of mikanolide which includes both α-1,10 and β-2,3-oxirane rings. The α- and quasiaxial nature of the C-3 carbon oxygen bond is further supported by the facile hydrogenolysis of **1** or **2** to **3**.[33a] Hydrogenation of the 4,5 double bond of **1** and **12** should proceed from the convex or β face and produce the α attachment of the carbonyl group at C-4 depicted in the formulas of the reduction products. Further work is in progress to verify these conclusions.

Miscandenin.—Although paucity of this substance, the least polar of the *M. scandens* constituents, precluded extensive chemical studies, evidence, based mainly on physical measurements, was acquired which supported its formulation as the interesting structure **17**.

At the outset the spectral data disclosed that miscandenin was structurally different from the mikanolide group of lactones. Thus the infrared spectrum, in addition to displaying the usual intense bands at 1770 and 1760 cm^{-1} characteristic of the dilactone functions of A and B, also displayed equally intense bands at 1680 and 1658 cm^{-1} which were too strong to be attributed to ordinary double bonds. Their position and intensity was, however, consistent with the presence of two enol ether functions as in **17**.[34-36] Furthermore the uv spectrum exhibited not only high intensity end absorption at 203 nm (ϵ 11900) due to an α,β-unsaturated lactone chromophore, but also a lower intensity maximum at 263 nm (ϵ 5640).

The nmr spectrum (Table II) of miscandenin was in complete accord with its formulation as **17** exclusive of stereochemistry. The assignments were confirmed

(32) (a) Owing to overlap of H-1 and H-9 signals in the nmr spectrum of **4a**, H-1 and H-9 had to be integrated together. The 13% figure assumes that there was no significant enhancement in the intensity of the H-9 resonance on irradiation of H-5. This could be demonstrated for **14b**. In the latter, the conformational difference referred to earlier[31] appeared to be responsible for the observation that irradiation at the frequency of H-5 produced a 12% increase in the integrated intensity of the combined H-1 and H-3 signals,[32b] but only relatively small enhancements (approximately 6% each) in the intensities of H-8 and H-6. However, the *cis* orientation of H-1 and H-8 in **14b** (and therefore in **12**) was conclusively demonstrated by irradiation at the frequency of H-1 which produced a 35% increase in the integrated intensity of the H-8 signal. (b) Based on the assumption that the H-13b signal which overlaps H-1 nd H-3 was not enhanced significantly.

(33) (a) Hydrogenolysis of allylic-type carbon–oxygen bonds proceeds when the bond is quasiaxial.[33b] (b) T. B. H. McMurry and R. C. Mollan, *J. Chem. Soc.*, 1619 (1969); A. Giger, M. Fetizon, J. Henniker, and L. Jaque, *Compt. Rend.*, **251**, 2194 (1960).

(34) The absence of one or more α,β-unsaturated ketone function implicit in this assumption was based on the uv spectrum (*vide infra*) and the ORD curve which showed no Cotton effect in the 290-nm region.

(35) See, for example, F. E. Bader, *Helv. Chim. Acta*, **36**, 215 (1953), for a discussion of ir spectra of representative compounds containing simple enol ethers and the grouping ROOC=CO–.

(36) The band at 1658 cm^{-1} was stronger than the band at 1680 cm^{-1}, probably owing to the superposition of the enol ether band on the absorption of the exocyclic double bond conjugated with the lactone function.

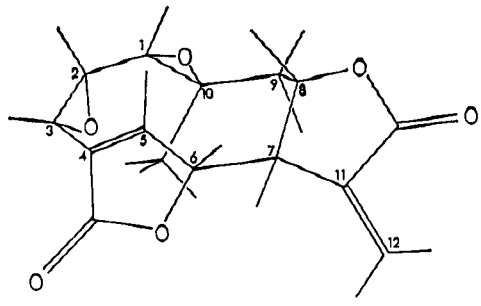
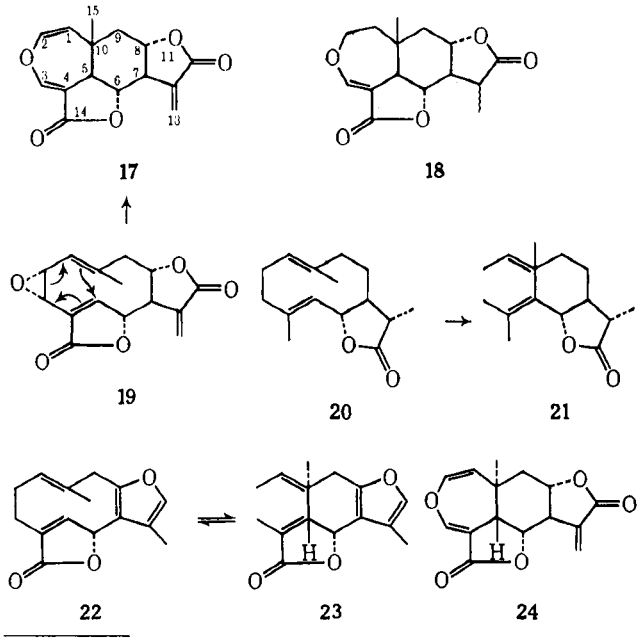


Figure 1.—Model of mikanolide.

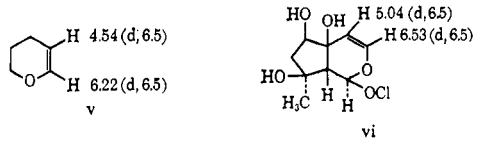
by double-resonance experiments. Chemical shifts of H-2 and H-1 were in good agreement with chemical shifts of α and β protons in enol ethers, the 8.5-Hz coupling constant being in excellent accord with a seven-numbered cyclic enol ether group,[37] but inconsistent with their incorporation into a smaller ring.[38] H-3 was shown to be allylically coupled to H-5 (J = 3.5 Hz) and H-6 was vicinally coupled to H-5 (J = 7 Hz) and H-7 (J = 10 Hz). As usual the latter[41] was allylically coupled to H_{13a} and H_{13b} (J = 3.5 Hz) and vicinally coupled to H-8 (J = 11 Hz). In turn H-8 was coupled to a two-proton multiplet centered at 1.9 (H-9, $J_{8,9a}$ = 3.5, $J_{8,9b}$ = 11 Hz).[42]



17 18 19 20 21 22 23 24

(37) R. Nagarajan, L. L. Huckstep, D. H. Lively, D. C. DeLong, M. M. Marsh, and N. Neuss, *J. Amer. Chem. Soc.*, **90**, 2980 (1968).

(38) For example in 2,3-dihydropyran (v)[39] and harpagide (vi)[40] the coupling constant is 6.5 Hz.



v vi

(39) N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "NMR Spectra Catalog," Varian Associates, Palo Alto, Calif., 1965, Spectrum No. 111.

(40) H. Lichti and A. von Warburg, *Helv. Chim. Acta*, **49**, 1552 (1966); M. L. Scarpati and M. Guiso, *Tetrahedron*, **23**, 4709 (1967).

(41) At 220 MHz, the H-7 multiplet was clearly resolved into a triplet of triplets, with the larger couplings ($J_{6,7}$ and $J_{7,8}$) of approximately 10.5 and the smaller couplings ($J_{7,13}$) of 3–3.5 Hz.

(42) At 220 MHz the H-9 multiplet was resolved into the AB part of an ABX system where A (H-9a) was at 2.11 (dd, 11.5, 3), B (H-9b) was at 1.80 (t, 11.5), and X was obviously H-8. We are grateful to Mr. R. S. Sudol and Dr. D. W. Ovenall, Plastics Department, E. I. du Pont de Nemours and Co., Inc., for determining the spectrum.

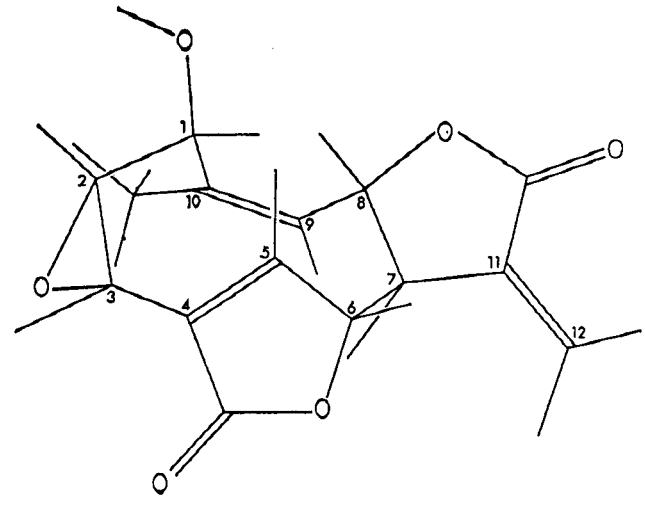
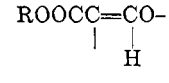


Figure 2.—Model of **4b**.

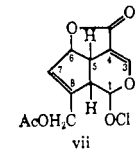
Hydrogenation of miscandenin resulted in the uptake of only 2 molar equiv of hydrogen and the formation of a tetrahydro derivative **18** whose infrared spectrum had retained one of the two intense enol ether bands. In the ultraviolet spectrum, the high intensity end absorption of **17** had disappeared and the long-wavelength band had experienced a hypsochromic shift to 248 nm (ϵ 7990). The nmr spectrum of **18** (Table II) reflected the changes which would be expected on reduction of partial structure A. Furthermore, the AB system of **17** representing H-1 and H-2 had disappeared, but the signal of the vinyl proton at lowest field (H-3) remained and the chemical shifts of H-5 and H-6 were not affected. Thus the nmr spectrum of the reduction product was in good agreement with its formulation as **18**.[43]

That the reduction of miscandenin stopped at the tetrahydro stage was not surprising in view of the observation that the grouping



is resistant to hydrogenation.[45] The absence of hydrogenolysis, in contrast to our experience with mikanolide (*vide supra*), further supports formula **17** for miscandenin which contains no allylic carbon–oxygen bond subject to hydrogenolysis. It is also eminently plausible on biogenetic grounds since it can be rationalized as arising from a hypothetical precursor, 1,10-deoxymikanolide (**19**), by a Cope rearrangement similar to the dihydrocostunolide–saussurea lactone

(43) Comparison of the nmr spectrum of **18** with that of asperuloside (vii),[44] which has H-3 at 7.20 (n d), H-5 at 3.48 (td), and H-6 at 5.49 (d, coupled here to H-5), is instructive.



vii

(44) L. H. Briggs, B. F. Cain, D. W. LeQuesne, and J. N. Shoolery, *Tetrahedron Lett.*, 69 (1963).

(45) See, for example, O. Halpern and H. Schmid, *Helv. Chim. Acta*, **41**, 1109 (1958).

(20 → 21)[46] and linderalactone–isolinderalactone (22 ⇄ 23) interconversions[47] which yield lactones belonging to the elemane class of sesquiterpenes.[50] The present situation is, however, slightly unusual in that the postulated Cope rearrangement would also involve a divinyloxirane–4,5-dihydrooxepine transformation of the type observed in the synthesis of 4,5-dihydrooxepine from *cis*-1,2-divinylethylene oxide.[51]

If, in analogy with the stereochemistry deduced for isabelin,[29] we assume a *trans*-$\Delta^{1(10)}$ bond for the hypothetical precursor **19**, orbital symmetry rules[52] require formation of a *trans*-A/B-fused elemadiene system, either C-10 methyl β, H-5 α, or the reverse.[53] If one makes the further highly plausible assumption that the stereochemistry of **19**, and therefore that of miscandenin, at C-6 and C-8 is identical with that of **1**, **12**, **15**, and **16**, construction of the almost impossibly strained model of **24** with C-10 methyl α, H-5 β, and ring B in a somewhat deformed chair leads to an implausible C-7 axial side chain and dihedral angles of approximately 30° for H-5,H-6 and 80° for H-6,H-7. This is incompatible with the observed coupling constants (7 and 10.5 Hz).[56] On the other hand, in the still strained model of **17**, with ring B in a chair, the all-*trans* relationship of H-5, H-6, and H-7 leads to dihedral angles of approximately 165° which harmonize with the H-5,H-6 and H-6,H-7 coupling constants.

Although all previously cited evidence fits in well with structure **17** for miscandenin, the ultraviolet maximum at 263 nm appears to be somewhat anomalous. The bathochromic shift of 15 nm relative to tetrahydromiscandenin is possibly due to an interaction, enforced by the presence of two fused rings, between the two enol ether double bonds, one of which is conjugated.[57] The ultraviolet maximum of tetrahydromiscandenin at 248 nm is also found at somewhat longer wavelength than that of model compounds possessing basically the same chromophore, though in the model compounds a carbalkoxy group has replaced the lactone ring or the stereochemistry differs.[59] This could account for the bathochromic shift.

Experimental Section[62]

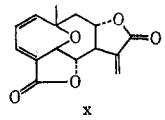
Isolation of Lactones from *Mikania Scandens* (L.) Willd.—The results of two typical large-scale extractions are described. Miscandenin could not be isolated from every collection.

A.—Above-ground material, dry wt 39 lb, collected by R. Lazor and J. Lazor in Sept 1967 in Wakulla County, Fla., along the Wakulla River 5 miles south of the upper bridge at the Wakulla Springs Wildlife Sanctuary, Wakulla County, Fla. (voucher 818-R1 on deposit in the Florida State University herbarium) was extracted with chloroform and worked up in the usual fashion.[63] The crude semisolid gum on being triturated with 300 ml of benzene–chloroform (1:1) deposited 5.2 g of solid which was a mixture of mikanolide (30%), dihydromikanolide (60%), and scandenolide (10%). The filtrate was concentrated to give 89 g of gum which was dissolved in the minimum of benzene–chloroform (1:1) and absorbed on 1.2 kg of silicic acid (Mallinckrodt, 100 mesh) packed in benzene. The column was eluted in the sequence shown below, 1:1 fractions being collected. In each case the solvents were evaporated and the residue was recrystallized from chloroform (or acetone)–ether–petroleum ether and monitored by tlc and nmr. The results are reported in Table V.

TABLE V[a]

Fractions	Eluent	Constituents	Wt, g
1–8	Benzene		
9–20	Benzene–chloroform (4:1)	Gummy (several spots)	
21–32	Benzene–chloroform (3:2)	Gummy (several spots)	
33–40	Benzene–chloroform (3:1)	MK	0.8
41–43	Benzene–chloroform (3:1)	MK + 10% DHMK	1.2
44–45	Benzene–chloroform (3:1)	MK + DHMK (1:1)	0.5
46–48	Benzene–chloroform (3:1)	DHMK + 10% MK	0.9
49–51	Benzene–chloroform (3:1)	DHMK	0.45
52–54	Benzene–chloroform (3:1)	DHMK (60%) + SC (40%)	0.35
55	Benzene–chloroform (3:1)	SC + DHSC (1:1)	0.3
Later	More polar	Noncrystallizable gums	

[a] The following abbreviations are used: MK, mikanolide; DHMK, dihydromikanolide; SC, scandenolide; DHSC, dihydroscandenolide.



x

(46) A. S. Rao, A. Paul, D. Sadgopal, and S. C. Bhattacharya, *Tetrahedron*, **13**, 318 (1961). The depicted *trans* stereochemistry of the $\Delta^{1(10)}$ bond of dihydrocostunolide is based on the conclusions of M. Suchy, V. Herout, and F. Sorm, *Collect. Czech. Chem. Commun.*, **31**, 2899 (1966).

(47) K. Takeda, H. Minato, and M. Ishikawa, *J. Chem. Soc.*, 4578 (1964); K. Takeda, I. Horibe, and H. Minato, *Chem. Commun.*, 378 (1968). *trans* stereochemistry of the $\Delta^{1(10)}$ bond of **22** was based on the failure to observe an NOE in the H-1 resonance on irradiation at the frequency of the C-10 methyl group[48] and on the facility with which isomerization to **23** occurred.[48] However, sericenin in which the double bond appears to be *cis* undergoes facile isomerization to isosericenin.[49]

(48) K. Takeda, I. Horibe, M. Teraoka, and H. Minato, *ibid.*, 637, 940 (1968).

(49) N. Hayashi, S. Hayashi, and T. Matsuura, *Tetrahedron Lett.*, 4957 (1968).

(50) W. Parker, J. S. Roberts, and R. Ramage, *Quart. Rev.*, **21**, 331 (1967).

(51) R. A. Braun, *J. Org. Chem.*, **28**, 1383 (1963); E. L. Stogryn, M. H. Gianni, and A. J. Passanante, *ibid.*, **29**, 1275 (1964). See also E. Vogel and H. Günther, *Angew. Chem.*, **79**, 429 (1967).

(52) R. B. Woodward and R. Hoffmann, *J. Amer. Chem. Soc.*, **89**, 4389 (1965).

(53) Although isomerization of germacranolides has so far led to isolation of only one of the two possible *trans*-fused elemanolides,[46,47,54] recent work indicates that the germacradiene–elemadiene rearrangement may, in certain cases, lead to both *trans*-fused elemadienes.[55]

(54) N. H. Fischer, T. J. Mabry, and H. B. Kagan, *Tetrahedron*, **24**, 4091 (1968).

(55) K. Morikawa and Y. Hirose, *Tetrahedron Lett.*, 2899 (1968); 869 (1969).

(56) With ring B in a deformed, but nevertheless apparently quite unfavorable boat conformation, the dihedral angles are much more satisfactory (~10 and 180°).

(57) The anomalous maximum near 213 nm in the spectra of germacrone, dihydrocostunolide, and similar compounds has been assigned to interaction between the endocyclic double bonds in the germacra-1,5-diene system.[58] In the present case, models suggest that the presence of the rigid endocyclic γ-lactone system and the (probable) *trans* fusion of ring B may impose geometrical restrictions on the relative orientation of the double bonds not present in 4,5-dihydrooxepin[51] which exhibits no uv absorption >200 nm. The alternative formulation x for miscandenin would account for the uv maximum more conventionally and also for the decoupling data, but is excluded on the basis of the AB system present in the nmr spectrum, the ir spectrum, the reduction experiments, and the properties of tetrahydromiscandenin.

(58) F. Sorm in "Progress in the Chemistry of Organic Natural Products," Vol. 19, L. Zechmeister, Ed., Springer Verlag, Vienna, 1961, p 1.

(59) *Cf.* asperuloside, 234.5 nm (ε 6760);[44] 6-isopropyl-3-carbomethoxy-5,6-dihydropyran, 240 nm (ε 13,500);[60] loganin, 236 nm (ε 10,900);[61] and tetrahydrodeoxyplumieride, 236 nm (ε 4075).[45]

(60) F. Korte, K. H. Büchel, and L. Schiffer, *Chem. Ber.*, **91**, 759 (1958).

(61) K. Seth, E. Ramstad, and J. Wolinsky, *Tetrahedron Lett.*, 394 (1961).

(62) Melting points are uncorrected; rotations were run in dioxane; ultraviolet spectra were run in 95% ethanol on a Cary Model 14 recording spectrophotometer unless otherwise stated; and infrared spectra were run as Nujol mulls on Perkin-Elmer 257 or 521 recording spectrophotometers. Tlc chromatograms were carried out on microslides coated with silica gel G, using chloroform–ethyl acetate–methanol (5:1:0.2) for development. Spots were detected by spraying with concentrated sulfuric acid followed by heating. Petroleum ether was low boiling (30–60°). Analyses were performed by Dr. F. Pascher, Bonn, Germany.

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

TABLE VI[a]

Fractions	Eluent	Constituents	Wt, g
1-10	Benzene		
11-22	Benzene-chloroform (4:1)	Gum	
23-39	Benzene-chloroform (3:1)	Gum	
40-43	Benzene-chloroform (3:2)	MSC	0.5
44	Benzene-chloroform (3:2)	MSC + MK (7:3)	0.05
45-55	Benzene-chloroform (3:2)	MK	3.5
56	Benzene-chloroform (3:2)	MK + DHMK (7:3)	0.4
57-59	Benzene-chloroform (3:2)	MK + DHMK (1:1)	1.0
60	Benzene-chloroform (3:2)	MK + DHMK (3:7)	0.4
61	Benzene-chloroform (3:2)	DHMK	0.38
62	Benzene-chloroform (3:2)	DHMK + DOMK (4:1)	0.3
63	Benzene-chloroform (3:2)	DHMK + DOMK (1:1)	0.25
64-68[b]	Benzene-chloroform (3:2)	DOMK + SC (1:1) DHMK (trace)	1.1
69-70	Benzene-chloroform (3:2)	DOMK + 10% SC	0.9
71	Benzene-chloroform (3:2)	DOMK + SC (6:4)	0.32
72	Benzene-chloroform (3:2)	DOMK + SC (1:1)	0.3
73	Benzene-chloroform (3:2)	DOMK + SC (1:2)	0.3
74	Benzene-chloroform (3:2)	SC + 10% DOMK	0.25
75-86	Benzene-chloroform (3:2)	SC	2.3
87-88	Benzene-chloroform (3:2)	SC + 10% DHSC	0.25
89-91	Benzene-chloroform (3:2)	SC + DHSC (7:3)	0.1
92-93	Benzene-chloroform (3:2)	SC + DHSC (1:1)	0.1
94	Benzene-chloroform (3:2)	SC + DHSC (1:4)	0.05
95-97	Benzene-chloroform (2:3)		
98-102	Benzene-chloroform (2:3)	DHSC	0.4
103-110	Benzene-chloroform (2:3)		
Later	More polar	Noncrystallizable gums	

[a] MK, mikanolide; DHMK, dihydromikanolide; MSC, miscandenin; DOMK, deoxymikanolide; SC, scandenolide; DHSC, dihydroscandenolide. [b] Erratic elution pattern.

B.—Dry plant material, 45 lb, collected by R. Lazor, J. Lazor, and K. Blum in Sept 1967 at the Ocklocknee River and U. S. 90 west of Tallahassee, Leon County, Fla. (voucher 821-R1 on deposit in the Florida State University herbarium) was extracted in the usual manner. The crude semisolid material furnished 7.5 g of a mixture of mikanolide (30%), dihydromikanolide (60%), and scandenolide (10%) on trituration with 200 ml of warm benzene. The filtrate gave 102 g of gum which was chromatographed as described in part A. The results are given in Table VI.

Miscandenin.—Recrystallization of fractions 40–43 in B from methylene chloride–ether or acetone–hexane afforded colorless needles: mp* 232–235°;[64] $[\alpha]^{24}D$ −181.4° (*c* 1.02, $CHCl_3$); λ_{max} 203 nm (ϵ 11,910) and 263 (5640); ir 1770, 1760, 1682, and 1658 cm^{-1}; mol wt (mass spectrum) 274.

Anal. Calcd for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15; O, 29.16. Found: C, 66.05; H, 5.03; O, 29.32.

Mikanolide.—Recrystallization of fractions 33–40 in A and 45–55 in B from acetone–hexane gave colorless rhombs: mp* 226–228°; $[\alpha]^{24}D$ +53.4° (*c* 1.12); λ_{max} 206 nm (ϵ 16,700); ir (KBr) 1767, 1752, 1666, and 1630 (sh) cm^{-1}; mol wt (mass spectrum) 200. Acetylation of mikanolide with acetic anhydride–pyridine resulted in recovery of starting material.

Anal. Calcd for $C_{15}H_{14}O_6$: C, 62.06; H, 4.86; O, 33.07. Found: C, 61.90; H, 5.37; O, 32.78.

Dihydromikanolide.—Recrystallization of fractions 49–51 in A and 61 in B from acetone–hexane afforded colorless prisms: mp 240–244° (complete liquefaction with gas evolution at 250–254°); $[\alpha]^{24}D$ +91.1° (*c* 0.47); ir (KBr) 1760 (double intensity) and 1650 cm^{-1}.

Anal. Calcd for $C_{15}H_{16}O_6$: C, 61.53; H, 5.58. Found: C, 61.77; H, 5.54.

Deoxymikanolide.—Repeated recrystallization of fractions 69–70 in B from acetone–hexane and acetone–diisopropyl ether afforded colorless plates and prisms: mp 198–200°; $[\alpha]^{25}D$ +98.9° (*c* 1.63); λ_{max} 211 nm (ϵ 12,880); ir bands at 1764, 1752, 1662, and 1654 cm^{-1}; mol wt (mass spectrum) 276.

Anal. Calcd for $C_{15}H_{16}O_5$: C, 65.22; H, 5.80; O, 28.98. Found: C, 65.17; H, 5.72; O, 29.17.

Scandenolide.—A single recrystallization of fractions 75–86 in B from acetone–isopropyl ether gave colorless needles: mp* 230–234°; $[\alpha]^{25}D$ +62.0° (*c* 1.11); λ_{max} 209 nm (ϵ 15,250), ir 1770, 1747, 1739, and 1657; mol wt (mass spectrum) 334 (weak), 294 (molecular ion − ketene).

Anal. Calcd for $C_{17}H_{18}O_7$: C, 61.08; H, 5.39; O, 33.54. Found: C, 61.15; H, 5.49; O, 33.40.

Dihydroscandenolide.—A single recrystallization of fractions 98–110 in B from acetone–hexane gave colorless prisms: mp 278–280° dec; $[\alpha]^{25}D$ +83.3° (*c* 0.54); λ_{max} 210 nm (ϵ 8680); ir 1778, 1742, and 1660 cm^{-1}.

Anal. Calcd for $C_{17}H_{20}O_7$: C, 60.71; H, 5.95; O, 33.34. Found: C, 60.80; H, 5.94; O, 33.36.

Separation of Mixed Fractions.—Mixtures in which one component was predominant, *e.g.*, fractions 41–43 and 46–48 in A, or 69–70, 74, and 87–88 in B, could be purified by repeated recrystallization. Mixtures in which the components were represented equally or nearly so were amenable to separation by a triangular recrystallization scheme from acetone–hexane. For example, fractions 44–45 and 55 in A, and 57–59, 63, and 71 were separated in this manner, the more sparingly soluble components (dihydromikanolide or dihydroscandenolide) being obtainable in almost pure form in the crop IC or ID stage.[65] The second component frequently could be obtained in pure form as crop IIC or IID. Three-component fractions could not be separated in this way and attempts to separate them by chromatography (column and preparative tlc) were unsuccessful.

Double Irradiation Studies on Mikanolide and Scandenolide.—The experiments on mikanolide, for which we are indebted to Mr. R. S. Sudol and Dr. D. W. Ovenall, were carried out on a Varian HA-100 nmr spectrometer in DMSO-d_6 solution. First, H-7 was located among the three-proton cluster of peaks centered around 3.4 ppm. Thus irradiation of either of the doublets at 6.20 and 5.29 ppm (H-13a and H-13b) effected simplification of this region. Conversely, irradiation at 3.4 ppm collapsed the doublets into singlets and also collapsed the 4.72 ppm multiplet (H-8, this

(64) Melting points which are starred were not sharp. The reported temperatures indicate the range in which the compound lost its crystallive form and collapsed to a glass when introduced at 200°. Complete liquefaction was not observed below 300°.

(65) R. S. Tipson in "Technique of Organic Chemistry," Vol. 3, A. Weissberger, Ed., Interscience Publishers, Inc., New York, N. Y., 1950, p 424.

was resolved into an octet at 100 MHz) into a quartet (10.5, 5.0) by removing an 8.5-Hz ($J_{7,8}$) coupling. Conversely, the 3.4-ppm multiplet was simplified by irradiation at 4.72.

The observation that H-8 was an octet required that it be flanked by a methylene group. This was proved as follows. Irradiation at 4.72 ppm (H-8) also collapsed two split doublets (H-9a and 9b, not resolved at 60 MHz) at 2.18 (10.5, 12.5) and 1.85 ppm (12.5, 5.0) into a pair of doublets separated by 12.5 Hz each. Irradiation at 2.0 ppm (center of H-9) caused collapse of the H-6 octet to a doublet (8.5). Hence $J_{8,9a} = 10.5$, $J_{8,9b} = 5.0$, $J_{9a,9b} = |12.5|$ Hz. With all couplings of H-9a and H-9b accounted for, the fourth bond of C-9 must be linked to a quaternary center as in C.

The environment of H-5 and H-6 was next clarified. Irradiation of the narrowly split doublet (1.7) of H-5 at 7.56 ppm converted the multiplet of H-6 at 5.42 ppm (apparent quintet) into a doublet of doublets (4.2, 1.1). Conversely, irradiation of H-6 collapsed H-5, removed the smaller coupling from the doublet of doublets of H-3 at 3.96 ppm (3.5, 1.1), and simplified the 3.4-ppm multiplet containing H-7 and two other protons now identifiable as H-1 and H-2. The converse was also true. Thus irradiation at 3.96 ppm perturbed the signal of H-6 and irradiation at 3.4 ppm collapsed H-6 into a narrow doublet of doublets whose lines were separated by 1.7 ($J_{5,6}$) and 1.1 Hz ($J_{3,6}$). Hence $J_{6,7}$ was 4.2 Hz.

Irradiation of the signal of H-3 perturbed the H-6 multiplet and affected the signals in the 3.4-ppm region, therefore known to contain H-2. Conversely, irradiation at 3.4 ppm removed the larger coupling (3.5 Hz) from the signal of H-3. Hence $J_{2,3}$ was 3.5 Hz.[66]

Decoupling experiments on scandenolide were carried out at 90 MHz in DMSO-d_6 solution. The following coupling constants were observed: $J_{1,2a} = 11.7$, $J_{1,2b} = 2$, $J_{2a,2b} = |14.5|$, $J_{2a,3} = 3.6$, $J_{3,5} < 0.5$, $J_{6,7} \sim 1$, $J_{7,8} = 3$, $J_{7,13a} = 3.5$, $J_{7,13b} = 3$, $J_{8,9a} = 4.7$, $J_{8,9b} = 8.9$ Hz. Owing to signal overlap it was not possible to determine $J_{2b,3}$, $J_{3,6}$ (less than 1 Hz), and $J_{5,6}$ (less than 1 Hz).

Ozonolysis of Mikanolide.—A solution of 100 mg of **1** in 15 ml of acetic acid was ozonized for 30 min at 0°, diluted with water, and steam distilled into an ice-cooled aqueous solution of dimedone. On standing 20 mg of the dimedone derivative of formaldehyde precipitated, mp and mmp 181–183°.

Hydrogenation of Mikanolide. A.—A solution of 145 mg of **1** in 40 ml of ethyl acetate was hydrogenated in the presence of 125 mg of 5% Pd–$CaCO_3$ at atmospheric pressure. The initially rapid uptake of hydrogen ceased after the consumption of 1 molar equiv. The solution was filtered and evaporated. The residue (single spot on tlc) was recrystallized from acetone–hexane, yield 120 mg of dihydromikanolide identical in all respects with material isolated from the plant.

B.—A solution of 145 mg of **1** in 40 ml of ethyl acetate was hydrogenated in the presence of 145 mg of 10% Pd–C catalyst at atmospheric pressure. Hydrogen uptake ceased after the absorption of *ca.* 2.5 molar equiv. The solution was filtered and evaporated. The residue (three spots on tlc) from two such runs was taken up in the minimum amount of chloroform and absorbed on 60 g of silicic acid set in benzene–chloroform (1:1). Elution with chloroform (10-ml fractions) gave the following results: fractions 1–6 (after elution started), 42 mg of tetrahydromikanolide (**3**); fraction 7, 18 mg of **3** and **10** (9:1); fraction 8, 6 mg of **3** and **10** (1:1); fraction 9, 4 mg of **3** and **10**; fraction 10–14, 16 mg of **10**; fractions 15–18, nothing; fractions 19–23 [chloroform–methanol (49:1)], 56 mg of **9b**.

Recrystallization of fractions 1–6 from acetone–hexane or ethyl acetate–petroleum ether furnished tetrahydromikanolide: mp 225–228°; $[\alpha]^{24}D$ +77.4° (*c* 0.8, $CHCl_3$); no uv absorption; ir (KBr) 1800 and 1755 cm^{-1}; nmr (pyridine-d_5) 4.88 (c, 2p, H-6 and H-8), 3.9 (c, 2p) and 3.2 (t, 1p) (H-1, H-2, and H-3), 1.55 (3p, C-10 methyl), and 1.3 ppm (dc, 7, C-11 methyl).

Anal. Calcd for $C_{15}H_{18}O_6$: C, 61.21; H, 6.17; O, 32.62. Found: C, 61.36; H, 6.32; O, 32.39.

Recrystallization of fractions 10–14 from ethyl acetate–hexane gave colorless needles of **10**: mp 220–224°, mmp 200–205° with **3**; $[\alpha]^{24}D$ +56.7° (*c* 1.0); no uv absorption; ir 1770 and 1760 cm^{-1}; nmr signals (pyridine-d_5) 4.78 (c, 2p, H-2 and H-8), 3.0–3.4 (c, 3p, H-1 and two other protons), 1.57 (3p, C-10 methyl), and 1.3 ppm (dc, 7, C-11 methyl).

Anal. Calcd for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19; O, 28.54. Found: C, 64.28; H, 7.23; O, 28.56.

Recrystallization of fractions 19–23 from acetone–hexane or ethyl acetate afforded **9b**: mp 243–245°; $[\alpha]^{24}D$ +58.5° (*c* 1.17); no uv absorption; ir 3468 and 1768 cm^{-1}; nmr (first figure refers to pyridine-d_5, second to $CDCl_3$) 3.43, 3.0 (sext) (H-2), 4.85, 4.66 (c, 2p) (H-6 and H-8), 3.73, 3.25 (d) (H-1), 2.05, 1.98 (3p, acetate), 1.72, 1.52 (3p, C-10 methyl), and 1.20 (d), 1.3 ppm (dc, 3p) (C-11 methyl).

Anal. Calcd for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80; O, 32.40. Found: C, 60.99; H, 6.74; O, 32.42.

Hydrogenation of Dihydromikanolide.—A solution of 292 mg of dihydromikanolide in 100 ml of ethyl acetate was hydrogenated at atmospheric pressure in the presence of 292 mg of 10% Pd–C. The initially rapid hydrogen uptake amounting to *ca.* 1 molar equiv during the first 0.5 hour was followed by slow uptake of *ca.* 0.7 molar equiv over several hours, after which consumption of hydrogen ceased. The product was worked up in the usual way: yield of **3**, 142 mg; yield of **10**, 28 mg; and yield of **9b**, 74 mg.

Acetylation of 80 mg of **9b** with acetic anhydride–pyridine and purification of the crude product by chromatography over 2 g of silicic acid (solvent and eluent chloroform) followed by recrystallization from ethyl acetate–petroleum ether afforded 58 mg of **9a**: mp 218–220°; $[\alpha]^{24}D$ +61.0° (*c* 1.0); nmr signals (pyridine-d_5) 4.88 (c, H-6 and H-8), 4.39 (sext, H-2), 3.7 (d, 3.5, H-1), 1.98 (acetate), 1.72 (C-10 methyl), and 1.20 ppm (d, 7, C-11 methyl).

Anal. Calcd for $C_{17}H_{22}O_7$: C, 60.64; H, 6.55; O, 33.10. Found: C, 60.46; H, 6.51; O, 32.94.

Rearrangement of Mikanolide to 4a and 4b.—A mixture of 0.4 g of mikanolide, 10 ml of acetic anhydride, and 0.2 g of *p*-toluenesulfonic acid was refluxed for 1 hr, taken to dryness *in vacuo*, diluted with ice water, and extracted with chloroform. Chromatography of the residue from the chloroform extract over 27 g of silicic acid (solvent and eluent chloroform) followed by recrystallization of the pure fractions from acetone–isopropyl ether afforded 256 mg of **4a**: mp 258–260°; $[\alpha]^{24}D$ −15.0° (*c* 0.94, $CHCl_3$); λ_{max} 206 nm (ϵ 19,600); ir bands at 1778, 1752, 1742, 1674, and 1661 cm^{-1}; nmr signals (pyridine-d_5) 7.8 (t, 1.5, H-5), 6.29 (d) and 5.82 (d) (3.2, exocyclic methylene), 5.72 (m, H-1 and H-5), 5.48 (dbr, 10, H-9), 5.23 (t, 10, H-8), 4.17 (m, H-3), 3.75 (t, 5, H-2), 1.96 (acetate), and 1.80 ppm (br, C-10 methyl). The same product was obtained in low yield on refluxing mikanolide with acetic acid.

Anal. Calcd for $C_{17}H_{16}O_7$: C, 61.44; H, 4.85; O, 33.70. Found: C, 61.53; H, 4.71; O, 33.93.

Hydrolysis of 106 mg of **4a** with 6 ml of a solution of 1.3 ml of concentrated HCl and 11 ml of methanol for 1 hr on the steam bath, evaporation to dryness repeatedly with methanol to remove hydrogen chloride vapors, and recrystallization of the residue from acetone–hexane afforded 82 mg of **4b**: mp 260–262°; $[\alpha]^{24}D$ +35.34° (*c* 0.96); λ_{max} 204 nm (ϵ 18,200); ir bands at 3560, 3460, 1760 (double intensity), 1670, and 1655 cm^{-1}; nmr signals (pyridine-d_5) at 7.6 (t, 1.5, H-5), 6.32 (d) and 5.82 (d) (3.2, exocyclic methylene), 5.77 (m, H-6), 5.37 (dbr, 10, H-9), 5.28 (t, 10, H-8), 4.88 (d, 5, H-1), 4.12 (m, H-3), 3.78 (t, 5, H-2), 3.5 (c, H-7), and 1.80 ppm (br, C-10 methyl).

Anal. Calcd for $C_{15}H_{14}O_6$: C, 62.06; H, 4.86; O, 33.07. Found: C, 62.38; H, 4.93; O, 32.72.

This substance was obtained more directly by hydrolysis of 120 mg of mikanolide with methanolic HCl in the manner described above. The yield was 78 mg. Reacetylation of 40 mg of **4b** with pyridine–acetic anhyride in the usual manner afforded 32 mg of **4a**.

Rearrangement of Dihydromikanolide to 5a and 5b.—Refluxing 300 mg of dihydromikanolide with acetic anhydride–*p*-toluenesulfonic acid in the manner described in the previous section and recrystallization of the product from acetone–isopropyl ether gave 186 mg of **5a**: mp 250–252°; λ_{max} 202 nm (ϵ 8600); ir bands at 1778, 1742 (double intensity), and 1660 cm^{-1}; nmr signals (pyridine-d_5) at 7.8 (t, 1.5, H-5), 5.65 (d, 5, H-1), 5.6 (m, H-6), 5.35 (dbr, 10, H-9), 5.2 (t, 10, H-8), 4.17 (m, H-3), 3.73 (t, 3, H-2), 2.75 (c, H-7 and H-11), 2.0 (acetate), 1.80 (br, C-10 methyl), and 1.35 ppm (d, C-11 methyl). The same product was obtained in lower yield by refluxing dihydromikanolide with acetic acid.

(66) The following points emerge from a comparison of the nmr spectra of **1** and **4a**. (1) While vicinal coupling exists between H-6 and H-7 in **1**, it is apparently absent in **4a**. (2) Whereas **4a** displays allylic coupling between H-3 and H-5 (J = 1.5 Hz), **1** does not. (3) Both **1** and **4a** exhibit homoallylic coupling between H-3 and H-6.

Anal. Calcd for $C_{17}H_{16}O_7$: C, 61.07; H, 5.43; O, 33.50. Found: C, 60.93; H, 5.78; O, 33.32.

Hydrolysis of 140 mg of **5a** with methanolic HCl as described for **4a** afforded 116 mg of **5b**: mp 281–283° after recrystallization from acetone–hexane; $[\alpha]^{24}D$ +18.7° (*c* 1.15); λ_{max} 203 nm (ϵ 8500); ir bands at 3520, 1782, 1750, and 1660 cm^{-1}; nmr signals (pyridine-d_5), at 7.65 (t, 1.5, H-5), 5.5 (m, H-6), 5.2 (H-8 and H-9), 4.85 (d, 5, H-1), 4.1 (m, H-3), 3.73 (t, 3, H-2), 2.7 (H-7 and H-11), 2.0 (br, C-10 methyl), and 1.33 ppm (d, 6.5, C-11 methyl). The same compound was obtained in 86-mg yield by refluxing 120 mg of dihydromikanolide with methanolic HCl.

Anal. Calcd for $C_{15}H_{16}O_6$: C, 61.64; H, 5.52; O, 32.85. Found: C, 61.73; H, 5.64; O, 32.68.

Catalytic hydrogenation of 318 mg of **4a** in 13 ml of ethyl acetate with Pd–$CaCO_3$ yielded 230 mg of **5a**.

Rearrangement of Tetrahydromikanolide to 6.—A mixture of 200 mg of tetrahydromikanolide, 100 mg of *p*-toluenesulfonic acid, and 6 ml of acetic anhydride was refluxed for 1 hr. Work-up in the usual manner followed by recrystallization from ethyl acetate afforded 98 mg of **6**: mp 265–267°; $[\alpha]^{24}D$ −67.4° (*c* 0.85); ir bands at 1770 (double intensity) and 1732 cm^{-1}.

Anal. Calcd for $C_{17}H_{20}O_7$: C, 60.71; H, 5.99; O, 33.30. Found: C, 61.11; H, 6.29; O, 32.72.

Oxidation of 4b.—A mixture of 100 mg of **4b** and 1.2 g of active maganese dioxide[67] was refluxed in 60 ml of dry benzene for 28 hr, filtered, and evaporated. The crystalline residue (tlc showed a single spot and 10% of starting material) was recrystallized three times from acetone–isopropyl ether and afforded 39 mg of **7** which did not melt below 320° and decomposed at 330–335°. It had $[\alpha]^{24}D$ −120° (*c*, 0.5); λ_{max} 206 and 318 nm (ϵ 17,350 and 200), λ_{max} (after subtraction of mikanolide chromophore) 250 and 318 nm (ϵ 3100 and 200); ir bands at 1778, 1768, 1700, 1675 (weak), and 1645 cm^{-1}.

Anal. Calcd for $C_{15}H_{12}O_6$: C, 62.50; H, 4.20; O, 33.30. Found: C, 62.63; H, 4.32; O, 32.99.

Oxidation of 5b.—Oxidation of 100 mg of **5b** with 1.0 g of active manganese dioxide in 50 ml of chloroform at room temperature for 36 hr and work-up in the manner described in the previous paragraph gave, after two recrystallizations from acetone, 56 mg of **8**: mp 315–318° dec; $[\alpha]^{24}D^{*}$ −92.9° (*c* 0.55); λ_{max} 206 and 318 nm (ϵ 9670 and 150), λ_{max} (after subtracting the chromophore of dihydromikanolide) 255 qnd 318 nm (ϵ 1200 and 150); ir bands at 1780, 1747, 1698, 1660, and 1635 cm^{-1}.

Anal. Calcd for $C_{15}H_{14}O_6$: C, 62.06; H, 4.86; O, 33.07. Found: C, 62.23; H, 4.83; O, 32.85.

Oxidation of 9a.—To an ice-cold stirred solution of 125 mg of **9a** in 10 ml of reagent grade acetone was added dropwise 0.4 ml of Jones reagent. The reaction mixture was stirred at ice temperature for 10 min and at room temperature for 20 min. Excess oxidant was destroyed by addition of a few drops of methanol. The mixture was diluted with ice water and the precipitate was filtered, washed, dried, and recrystallized from acetone. This afforded 68 mg of ketone **11**: mp 305–30°; λ_{max} 203 nm (ϵ 1275) and 285 nm (ϵ 68); ir bands at 1780, 1770, and 1715 cm^{-1}.

Anal. Calcd for $C_{15}H_{18}O_6$: C, 61.21; H, 6.17; O, 32.62. Found: C, 60.87; H, 6.24; O, 32.65.

An attempt to reduce **11** with chromous chloride in acetone–acetic acid solution resulted in recovery of starting material.

Hydrogenation of Scandenolide. A.—Hydrogenation of 100 mg of **12** with Pd–$CaCO_3$ catalyst in ethyl acetate in the manner described for mikanolide afforded after recrystallization 73 mg of **13** identical in all respects with material isolated from the plant.

B.—Hydrogenation of 130 mg of **12** with 80 mg of 10% Pd–C in 20 ml of ethyl acetate stopped after consumption of approximately 2 molar equiv of hydrogen (6 hr). Work-up in the usual manner afforded 128 mg of tetrahydroscandenolide (one spot on tlc) which was recrystallized from acetone and had mp 231–233°; $[\alpha]^{24}D$ +50.4° (*c* 0.64, dioxane); nmr peaks (DMSO-d_6) at 2.12 (acetate), 1.28 (C-10 methyl), and 1.16 ppm (C-11 methyl). The other signals could not be distinguished clearly.

Anal. Calcd for $C_{17}H_{22}O_7$: C, 60.64; H, 6.55; O, 33.10. Found: C, 60.52; H, 6.62; O, 33.28.

Rearrangement of Scandenolide to 14a.—A solution of 120 mg of scandenolide in 6 ml of methanolic hydrochloric acid (prepared from 5.4 ml of methanol and 0.6 ml of HCl) was refluxed for 1 hr and taken to dryness repeatedly after addition of methanol to remove HCl. The solid residue (single spot on tlc) was recrystallized from acetone and afforded 86 mg of the diol **14a**: mp 312–315°; ir bands (Nujol) at 3500, 1742 (double intensity), 1670, and 1652 cm^{-1}.

Anal. Calcd for $C_{15}H_{16}O_6$: C, 61.65; H, 5.48; O, 32.88. Found: C, 61.82; H, 5.45; O, 32.92.

Acetylation of 100 mg of **14a** with 2 ml of pyridine and 2 ml of acetic anhydride at 80° for 2 hr yielded, after recrystallization from ethyl acetate–petroleum ether, colorless needles of the diacetate **14b**, mp 204–206°.

Anal. Calcd for $C_{19}H_{20}O_8$: C, 60.64; H, 5.36; O, 34.01. Found: C, 60.71; H, 5.34; O, 34.08.

Preparation of Deoxymikanolide from Isabelin.—A solution of 95 mg of isabelin (**16**)[30] in 4 ml of chloroform was left with 62 mg of *m*-chloroperbenzoic acid in 2 ml of chloroform at room temperature for 2.5 hr. After the mixture was washed with saturated sodium bicarbonate solution and water, the organic layer was evaporated at reduced pressure to give 123 mg of solid which on recrystallization from acetone–hexane gave deoxymikanolide, mp 198–199.5°, identical in all respects with material isolated from the plant.

Tetrahydromiscandenin (18).—A solution of 100 mg of miscandenin in 25 ml of ethyl acetate was reduced at atmospheric pressure in the presence of 10% Pd–C. Two molar equivalents of hydrogen were rapidly absorbed during the first 25 min, after which hydrogen uptake ceased. After the usual work-up the solid residue (single spot on tlc) was recrystallized from ethyl acetate–petroleum ether. The product, 68 mg, had mp 188–190°, λ_{max} 248 nm (ϵ 8000), ir bands (Nujol) at 1755 and 1668 cm^{-1}.

Anal. Calcd for $C_{15}H_{18}O_5$: C, 64.73; H, 6.52; O, 28.75. Found: C, 64.35; H, 6.74; O, 29.04.

NOE Experiments.—These were carried out on a 90-Mc Bruker nmr spectrometer on degassed samples in $CHCl_3$–DMSO solution.

4a.—Reference: irradiation 240 Hz upfield from $CHCl_3$ signal, integrated intensity of C-10 methyl 95 ± 1 (average of three integrations), acetate 104 ± 2 (3, reference), ratio 0.91. Irradiation 257.4 Hz upfield (H-9), intergrated intensity of C-10 methyl 95 ± 1 (3), acetate 94 ± 2 (3, reference), ratio 1.01, hence 11% enhancement. Reference: irradiation 49.9 Hz from $CHCl_3$, integrated intensities H-6 73, H-8 84, H-1 + H-9 149, H-13a 69, H-13b 64 (average of five integrations). Irradiation 42.3 Hz upfield (H-5), integrated intensities (average of five integrations), H-6 61 (20% enhancement), H-8 67 (26%), H-1 + H-9 140 (13%), H-13a 66 (4%), H-13b 68 (−6%). H-2 and H-3 were also irradiated and an attempt was made to observe NOE's on H-1, H-5, H-6, H-8, H-9, and H-13, but all enhancements were less than 10%.

12.—Reference: irradiation 36.5 Hz downfield from $CHCl_3$ signal, integrated intensities (average of four integrations) H-1 74, H-3 + H-6 97, H-8 61, H-13 98. Irradiation 27.2 Hz downfield (H-5), integrated intensities (average of four), H-1 71 (−4%), H-3 + H-6 98 (1%), H-8 63 (5%), H-13 96 (−2%). Reference: irradiation 369.6 Hz upfield, integrated intensities (average of five) H-13 130, C-10 methyl 245. Irradiation 493.5 Hz upfield (H-1), integrated intensites (five), H-5 56, H-13 142. Irradiation 402.8 Hz upfield (H-8), integrated intensities (five), H-5 65 (16% enhancement), H-13 148 (4%).

14b.—Reference: irradiation 530 Hz downfield from TMS signal, integrated intensities (six), H-8 37, H-9 41. Irradiation 504.5 Hz downfield (H-1), integrated intensities (six) H-8 50 (34% enhancement), H-9 38 (−8%). Reference: irradiation 675.8 Hz downfield from TMS signal, integrated intensities (four), H-1, H-3 and H-13b 111, H-2 34, H-6 37, H-9 38. Irradiation 661.9 Hz downfield (H-5), integrated intensities (four), H-1, H-3 and H-13b 117 (6% total enhancement, 12% if limited to H-1 and H-3), H-2 37 (9%), H-6 39 (5%), H-7 31 (3%), H-8 37 (6%), H-9 38 (3%). Reference: irradiation 540.2 Hz downfield from TMS, integrated intensities (five), C-1 acetate 54, C-3 acetate 75, C-15 methyl 51. Irradiation 468.2 Hz downfield from TMS, integrated intensities (five), C-1 acetate 70 (11% enhancement), C-3 acetate 72 (−4%), C-15 methyl 56 (10%).

Isabelin (16).—Reference: irradiation 369.6 Hz downfield from TMS, integrated intensities (five), H-13 130, C-10 methyl 245. Irradiation 439.5 Hz fownfield (H-1), integrated intensities (five), H-13 141 (8.5% enhancement), C-10 methyl 247 (0.8%). Reference: irradiation 340.4 Hz downfield from TMS, integrated intensities (five), H-5 56, H-13 142. Irradiation 402.8 Hz down-

(67) J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen, and T. Walker, *J. Chem. Soc.*, 1094 (1952).

field, integrated intensities (five), H-5 65 (16% enhancement), H-13 148 (4%).

Registry No.—**1**, 23753-56-2; **2**, 23758-04-5; **3**, 17940-97-5; **4a**, 23758-06-7; **4b**, 23758-07-8; **5a**, 23758-08-9; **5b**, 23758-09-0; **6**, 23758-10-3; **7**, 23758-11-4; **8**, 23758-12-5; **9a**, 23829-41-6; **9b**, 23829-42-7; **10**, 23758-13-6; **11**, 23758-14-7; **12**, 23758-16-9; **13**, 23758-17-0; **14a**, 23758-18-1; **14b**, 23758-19-2; **15**, 23753-57-3; **17**, 23758-20-5; **18**, 23758-21-6; tetrahydroscandenolide, 23758-15-8.

Studies in the Ganglioside Series. IV. Preparation of 2,3-Di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose and Its Utilization in the Synthesis of Oligosaccharides[1]

DAVID SHAPIRO, Y. RABINSOHN, A. J. ACHER, AND A. DIVER-HABER

Department of Chemistry, The Weizmann Institute of Science, Rehovoth, Israel

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2,3-Di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (V) has been prepared by cyclization of phenyl 2,3,6-tri-*O*-acetyl-4-*O*-*t*-butyl-β-D-glucopyranoside (III) and removal from IV of the protecting *t*-butyl group by trifluoroacetic acid. Compound III was obtained by acid-catalyzed addition of 2-methylpropene to phenyl 2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (II). The use of V as aglycon in the Koenigs–Knorr reaction will permit the synthesis of oligosaccharides containing a glycosidic linkage at C-4 of glucose. This is demonstrated by the synthesis of lactose and of the aminosaccharide 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-glucopyranose (XIII).

The carbohydrate chain of the gangliosides comprises a tetrasaccharide in which galactose is attached to glucose by a 1 → 4 linkage.[2,3] It is well known that the C-4 hydroxyl group in the *C1* conformation of glucopyranose, although equatorially oriented, exhibits rather low reactivity. Richardson[4,5] has shown that the differential reactivity of the secondary hydroxyls in glucopyranosides is not solely dependent on the conformation. The 4-OH is also sterically hindered by adjacent substituents, especially by the 5-acyloxymethyl group. Because of these features of the glucose molecule, the synthesis of disaccharides of the lactose type has posed a problem ever since. Curtis and Jones,[6] using the open chain form of glucose, condensed 2,3,5,6-di-*O*-isopropylidene-D-glucose diethyl acetal with acetobromogalactose and obtained a mixture of mono- and disaccharides from which lactose could be separated by charcoal and paper chromatography.

During the course of our studies[7-9] on the gangliosides it became imperative to devise a suitably substituted glucose derivative in which the free C-4 hydroxyl would have enhanced reactivity. Earlier investigators recognized the synthetic value of 1,6-anhydro-hexopyranoses. In 1933 Freudenberg[10] coupled unsubstituted 1,6-anhydro-β-D-glucopyranose with acetobromoglucose and obtained a mixture from which cellobiose could be isolated in a 2% yield. Hudson[11] first synthesized lactose *via* its epimer (4-*O*-galactopyranosyl-β-D-mannopyranose), employing 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose as the aglycon. The presence in the mannose molecule of two neighboring *cis* hydroxyls offers a convenient means for selective substitution by the isopropylidene group. However, since the glucose molecule lacks this possibility, we explored a different route for the preparation of a 1,6-anhydro derivative in which the C-4 hydroxyl is free for reaction.

1,6-Anhydro-β-D-glucopyranose exists in the *1C* conformation. Although all of the hydroxyl groups are axially oriented, steric considerations indicate that those in positions 2 and 4 will react preferentially. The C-3 hydroxyl is the most hindered one, owing to the hemiacetal and anhydro rings, and to the C–C linkage at C-5.[12] Indeed, esterification with benzoyl chloride, tosyl chloride, or benzyl chloroformate was found to give high yields of the 2,4-diacyl derivatives,[12,13] and benzylation, even under drastic conditions, likewise produced the 2,4-dibenzyl derivative in appreciable amounts.[14]

We now report the synthesis of 2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (V) (Scheme I). The route adapted involves blocking of the C-4 hydroxyl by the *t*-butyl group. This group has been used in peptide synthesis for the protection of hydroxyamino acids and is conveniently introduced by acid-catalyzed addition of 2-methylpropene.[15-17] Except for one case, in which a similar reaction was carried out by a different method and under drastic conditions,[18] no attempt has been made to employ this olefin in carbohydrate chemistry. The acid-catalyzed reaction of *t*-butyl alcohol with glucose was reported to give preferentially the 6-*O*-derivative,[19] whereas the use of *t*-butyl bromide met with little success.[20]

(1) Supported by U. S. National Institutes of Health, PL 480, Agreement No. 425115.

(2) L. Svennerholm, *J. Lipid Res.*, **5**, 145 (1964).

(3) H. Wiegandt, *Angew. Chem. Int. Ed. Engl.*, **7**, 87 (1968).

(4) A. C. Richardson, *Chem. Commun.*, 104 (1965).

(5) J. M. Williams and A. C. Richardson, *Tetrahedron*, **23**, 1369 (1967).

(6) E. J. C. Curtis and J. K. Jones, *Can. J. Chem.*, **37**, 358 (1959).

(7) D. Shapiro, A. J. Acher, and E. S. Rachaman, *J. Org. Chem.*, **32**, 3767 (1967).

(8) A. J. Acher and D. Shapiro, *ibid.*, **34**, 2652 (1969).

(9) D. Shapiro and A. J. Acher, *ibid.*, **35**, 229 (1970).

(10) K. Freundenberg and W. Nagai, *Chem. Ber.*, **66**, 27 (1933).

(11) W. T. Haskins, R. M. Hann, and C. S. Hudson, *J. Amer. Chem. Soc.*, **64**, 1852 (1942).

(12) M. Černý, V. Gut, and J. Pacák, *Collect. Czech. Chem. Commun.*, **26**, 2542 (1961).

(13) R. W. Jeanloz, A. M. C. Rapin, and S. Hakomori, *J. Org. Chem.*, **26**, 3939 (1961).

(14) G. Zemplén, Z. Csürös, and S. Angyal, *Chem. Ber.*, **70**, 1848 (1937).

(15) H. C. Beyerman and J. S. Bontekoe, *Proc. Chem. Soc.*, 249 (1961).

(16) F. M. Callahan, G. W. Anderson, R. Paul, and J. E. Zimmerman, *J. Amer. Chem. Soc.*, **85**, 201 (1963).

(17) E. Wuensch and J. Jentsch, *Chem. Ber.*, **97**, 2490 (1964).

(18) V. Prey and F. Grundschober, *ibid.*, **95**, 1845 (1962).

(19) A. N. de Belder, E. J. Bourne, and H. Weigel, *Carbohyd. Res.*, **3**, 1 (1966).

(20) J. Kenner and G. N. Richards, *J. Chem. Soc.*, 3019 (1957).