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CONSTITUENTS OF *GAILLARDIA* SPECIES—IV

THE SESQUITERPENE LACTONES OF *GAILLARDIA FASTIGIATA* GREENE[1,2]

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Abstract—Structures have been established for three new sesquiterpene lactones, which were isolated from *Gaillardia Fastigiata* Greene, and for a previously reported[3] minor constituent of *G. pinnatifida* Torr.

IN EARLIER papers of this series[2-4] we reported the isolation and structure determination of pseudoguaianolides from collections of *Gaillardia pulchella* Foug., *G. megapotamica* (Spreng.) Baker, *G. multiceps* Greene, *G. pinnatifida* Torr. and *G. arizonica* Gray. We now describe the chemical investigation of *G. fastigiata* Greene,[5] which has yielded three new pseudoguaianolides and the flavone hispidulin (6-methoxy-5,7,4'-trihydroxyflavone).[9]

The yields of crystalline substances from *G. fastigiata*, which could be isolated only after extensive chromatography, were very small. A collection from the vicinity of Dallas, Texas, furnished two apparently isomeric lactones of formula $C_{20}H_{26}O_6$ in 0·04 and 0·07% yield which were named fastigilin A and fastigilin B. A large-scale collection from western Oklahoma furnished no fastigilin A, some fastigilin B, (0·002%), 0·005% of a new lactone $C_{20}H_{24}O_6$ which was named fastigilin C and 0·001% of hispidulin. In addition there was isolated in 0·007% yield a substance

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

[2] Previous paper, W. Herz, S. Rajappa, M. V. Lakshmikantham and John J. Schmid, *Tetrahedron* **22**, 693 (1966).

[3] W. Herz, K. Ueda and S. Inayama, *Tetrahedron* **19**, 483 (1963).

[4] W. Herz and S. Inayama, *Tetrahedron* **20**, 347 (1964).

[5] The taxonomic status of *Gaillardia*, section *Hollandia* is uncertain at the present. *G. fastigiata* Greene is generally recognized as a separate species.[6] However the distinction from *G. lanceolata* Michx. (*G. aestivalis* (Walt.) H. Rock[7]) is based primarily on the length of the peduncle and may be artificial although the existence of intraspecific variations is recognized.[8] These variations may be responsible for the difference in sesquiterpene lactone content encountered in the present work (Experimental). A phytochemical investigation of *G. aestivalis* now in progress may shed light on this matter.

[6] S. F. Biddulph, *Res. Studies, Wash, State Coll.* **12**, No. 4, 195 (1944).

[7] H. F. L. Rock, *Rhodora* **58**, 311 (1956).

[8] Private communication from Dr. W. P. Stoutamire, Cranbrook Institute of Science, Bloomfield Hills, Mich.

[9] W. Herz and Y. Sumi, *J. Org. Chem.* **29**, 3438 (1964). Whalley *et al.*[10] reported the presence of this flavone in a crude pigment mixture from *Digitalis lanata L.* but were unable to isolate it except in the form of derivatives.

[10] G. O. P. Doherty, N. B. Haynes and W. B. Whalley, *J. Chem. Soc.* 5577 (1963).

$C_{15}H_{16}O_6$, obtained previously[11] from several members of the tribe *Heliantheae*, family *Compositae*, which we have called mikanolide and which will be the subject of a separate study.

Fastigilin B, $C_{20}H_{26}O_6$, m.p. 259–261°, exhibited UV absorption at 222·5 mμ (ε 22200). The high intensity indicated the presence of two chromophores absorbing in the same region. IR bands at 3500, 1752, 1720, 1705, 1650 and 1585 cm^{-1} [12] suggested the presence of a hydroxyl group, a γ-lactone, an α,β-unsaturated cyclopentenone of the type found in ambrosin[13] and tenulin[14] and an unsaturated conjugated ester which because of the analytical values probably was an angelate, tiglate or senecioate.

Catalytic hydrogenation confirmed the presence of two double bonds. The product, tetrahydrofastigilin B (IIb) exhibited no UV absorption in the 210–250 mμ range and now displayed IR bands characteristic of a hydroxyl group, a γ-lactone, a cyclopentanone and a saturated ester. Acetylation of fastigilin B to Ic, and of IIb to IIc confirmed the presence of a hydroxyl group. That the latter was secondary and incorporated in a six-membered (or larger) ring was established by the facile oxidation of IIb to a dehydro derivative IIIb which exhibited a new ketone band at 1718 cm^{-1}.

The catalytic hydrogenation of fastigilin C, $C_{20}H_{24}O_6$, m.p. 197–199°, $[\alpha]_D$ −85·8°, to tetrahydrofastigilin B revealed the close relationship of the two natural products. That the extra double bond of fastigilin C indicated by the analytical data and an additional IR band at 1655 cm^{-1} was part of an exocyclic double bond conjugated with the lactone function was suggested by the enhanced UV absorption at 222·5 mμ ($\varepsilon = 29300$) and by ozonolysis which liberated formaldehyde as well as acetone. This last observation also confirmed that fastigilin B and C were senecioyl esters of sesquiterpene lactones.

The NMR spectra (Table 1) permitted verification of these assignments. First, fastigilin C (IVa), its acetate (IVb) and Ic exhibited the low-field doublets of doublets near 7·7 and 6·1 ppm characteristic of partial structure A which disappeared on reduction to IIb and IIc.[13,14] Secondly fastigilin C and IVb displayed the usual narrowly-split doublets of the conjugated methylene group of partial structure B[15] which in the spectrum of Ic and on reduction of IVa and IVb were replaced by the signal of a new secondary methyl group.

The presence of the senecioyl side chain was confirmed by a vinyl proton resonance at 5·5 ppm, which disappeared on reduction and by two narrowly-split vinyl methyl doublets, which moved upfield and then exhibited the usual 7 c/s splitting on reduction. Other signals occurred near 5·3 br (hydrogen on carbon carrying the ester function), 4·8–5·0 dd (H_d of B) and 3·6 c (intensity two protons, hydrogen on carbon carrying secondary hydroxyl and probably H_c of B). The identity of one component of this signal was clear from its behavior on acetylation (downfield shift in Ic and IVb) and oxidation (disappearance in IIIb). Furthermore the conversion of IIc to IIIb was

[11] W. Herz, unpublished.

[12] Frequency and clarity of these bands depend on the state of the sample and is specified in the Experimental.

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

[14] W. Herz, W. A. Rohde, K. Rabindran, P. Jayaraman and N. Viswanathan, *J. Amer. Chem. Soc.* **84**, 3857 (1962).

[15] W. Herz, A. Romo de Vivar, J. Romo and N. Viswanathan, *J. Amer. Chem. Soc.* **85**, 19 (1963).

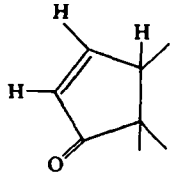
TABLE 1. NMR SPECTRA OF FASTIGILIN DERIVATIVES[a]

	H_3	H_2	H_6	H_7	H_8	H_9	H_{13}	C_5–Me	C_{10}–Me	C_{11}–Me	Misc.
Ia	7·75 dd (6, 2)	6·10 dd (6, 3)	5·45 br[b]		4·92 dd (6, 2)	3·5 c		1·02	1·38 d (6·5)	1·52 d (6·5)	6·1[c], 1·90 dq (6·5, 1)[d] 1·71 m (1·5)[d]
Ic	7·60 dd (7, 2)	6·0 dd (7, 3)	5·35 br[b]		4·88 dd (10, 2)	4·60 m		1·02	1·17 d (7)	1·50 d (6)	5·35 m[e], 3 c[f], 2·10 d (1·5)[g] 1·80 d (1·5)[g], 2·1[h]
IIc			5·30 br[b]		4·83 dbr (7)	3·5 dbr (7)		0·9[i]	1·15 d (6·5)	1·45 d (7)	0·9[i]
IId			5·30 br[j]		4·85 dd (10, 1·5)	5·0 dd (7, 1·5)		0·85	1·18 d (6·5)	1·45 d (6·5)	3·12[k], 0·92 d (7·5) 0·92 d (7·5)[l]
IIIb			5·28 br[b]		4·75 d (7)			0·98	1·18 d (6·5)	1·45 d (7)	0·9 d, 0·9 d[l]
IVa	7·72 dd (6, 1·5)	6·06 dd (6, 3)	5·27 br[b]	3·6 c	5·0 dd (7, 2·1)	3·63 c	6·42 d (2·5) 6·22 d (2)	0·98	1·36 d (6·5)		5·5[e], 3·18[f], 2·17 d (1)[g], 1·86 d (1)[g]
IVb	7·66 dd (7, 2)	6·04 dd (7, 3)	5·25 br[b]	3·7 c	4·76 (8, 2)	4·97 (9, 2)	6·40 d (2) 6·21 d (2)	1·01	1·23 d (7)		5·5 m[e], 3·15 c[f], 2·14[g], 2·14[h], 1·88 d (1)[g], 2·5 c[m]
IVc	7·75 dd (6·5, 2)	6·12 dd (6·5, 2)	5·66 d (8)	3·1 c	4·8 c	4·8 c	6·16 d (3) 5·82 d (3)	1·27	1·22 d (7)		3·1 c[f], 2·14[h], 1·95[h], 2·1[m]

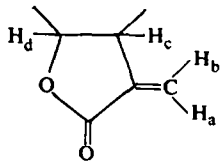
[a] Spectra were run in $CDCl_3$ solution on a Varian A-60 spectrometer, with tetramethylsilane serving as internal standard. Values are given in ppm. Numbers in parentheses correspond to line separations in c/s. All signals in first seven columns correspond to one proton, those in next three columns to three protons. Singlets are unmarked multiplets are described as follows: d doublet, q quartet, br broadened signal, c complex signal whose center is given, m multiplet.

[b] $W_{1/2}$ 3·5
[c] Vinyl H in angeloyl side chain
[d] Vinyl methyl in angeloyl chain
[e] Vinyl H in senecioyl side chain
[f] H_1
[g] Vinyl methyl in senecioyl side chain
[h] acetate
[i] three superimposed methyl signals
[j] $W_{1/2}$ 2·5
[k] mesylate
[l] methyls in side chain
[m] H_{10}

accompanied by a change in the multiplicity of the signal near 4·9 ppm which indicated that the secondary hydroxyl group flanked the lactone group as in C.



A



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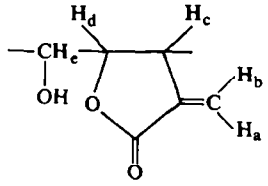

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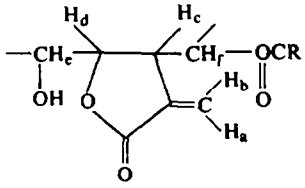

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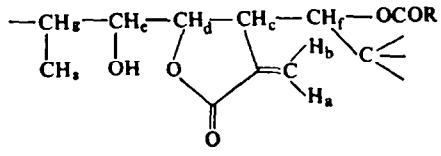


E

Consideration of A and C, coupled with the necessity of accommodating an esterified secondary alcohol function and a secondary and a tertiary methyl group (Table 1) led to formula IVa for fastigilin C and hence Ib for fastigilin B if it is assumed that the distribution of groups in the seven-membered ring follows the biogenetic isoprene rule. That these formulae are indeed correct was shown by double irradiation experiments which will now be detailed.

Irradiation of fastigilin C at 3·08 ppm reduced the signals assigned to H_2 and H_3 to doublets (J = 6). Conversely, irradiation at 7·72 ppm collapsed H_2 to a doublet (J = 3·1), while irradiation at 6·06 collapsed H_3 to a doublet, (J = 1·5), both experiments producing a change in the complex signal at 3·08 ppm which must therefore be assigned to H_1. This establishes partial formula A.

Irradiation at 3·63 ppm collapsed the doublets at 6·42 and 6·22 to singlets. Hence one of the two protons giving rise to this signal is allylically coupled to H_a and H_b and must be H_c, the other having been previously identified as H_e. Irradiation at 3·63 ppm (H_c and H_e) also collapsed the H_d multiplet at 5 ppm ($J_{H_cH_d}$, $J_{H_dH_e}$ = 7,2) to a singlet and reduced the broadened resonance at 5·27 ppm to a sharp singlet. This indicated that either H_c or H_e was vicinally coupled to the proton on carbon carrying the senecioyl function which in turn must adjoin a quaternary center. Conversely, irradiation at 5·0 (H_d) or at 5·27 (H_f) ppm effected simplification of the signals assigned to H_c and H_e.

Further clarification could be obtained by spin-decoupling certain signals in the NMR spectrum of IVb (Table 1). Irradiation at 3·7 ppm (H_c) collapsed the resonance at 4·76 ppm to a broad singlet, thus differentiating the H_d resonance from that of H_e which was found at 4·97 ppm and confirming $J_{H_cH_d}$ as 8 c/s. Irradiation at 74 c/s downfield from 1·23 ppm caused collapse of the methyl doublet which located the center of what eventually turned out to be H_{10} at 2·5 ppm. Irradiation at 2·5 ppm also removed the large coupling (J = 9) from the H_e resonance at 4·97 ppm. This permits expansion of D to E, where $J_{H_dH_e}$ = 2 and $J_{H_eH_g}$ = 9, and leads to IVa as the sole formula for fastigilin C compatible with the evidence.

The physical properties of fastigilin A, $C_{20}H_{26}O_6$, m.p. 175–177°, [α] −81·6°, λ_{max} 222 mμ (ε = 18000), showed that it was closely related to fastigilin B. IR bands at 3600, 1778, 1722 (double intensity), 1650 and 1590 cm^{-1} coupled with the analytical values indicated the presence of a free hydroxyl group, a γ-lactone, an α,β-unsaturated cyclopentenone and an unsaturated five-carbon ester side chain. Catalytic hydrogeneration to IIa confirmed the presence of two double bonds, oxidation of IIa to IIIa the presence of a secondary hydroxyl group. The amount of material available was not sufficient to permit experimental correlation of fastigilin A with fastigilin B or C, but the NMR spectrum (Table 1) established the presence of an angeloyl rather than a senecioyl side chain at C_6 and indicated that, because of the similarity of chemical shifts and coupling constants, the stereochemistry of fastigilin A corresponded to that of fastigilin B at all centers except perhaps at C_{11}.

As regards absolute configuration, the ORD curves of fastigilin C and its reduction product IIb corresponded in shape and sign of Cotton effect to those of *trans*-fused pseudoguaianolides of established stereochemistry (C_5-methyl β, H_1 α).[16–22] Specifically, fastigilin C exhibited a pronounced negative Cotton effect of an amplitude comparable to that of helenalin (V, R = H)[16] although displaced entirely to negative values (Experimental), while tetrahydrofastigilin B had the typical strongly positive Cotton effect (a = 80·2) of tetrahydrohelenalin and its congeners.[16] If now the assumption is made that the C_7-side chain of the components of *G. fastigiata* is equatorial and β, as in all other sesquiterpene lactones of known configuration, the analogy between the coupling constants observed in this work and those of helenalin derivatives (V) leads to the tentative formulation of fastigilin C as VI, where the only remaining uncertainty is the orientation of the hydroxyl group at C_9.

The oxidation pattern demonstrated for the fastigilins is the same as that assigned earlier[2] to a minor constituent of *G. pinnatifida* Torr., for which formula IVc was proposed on the basis of spectral data. This formula has now been verified by double irradiation experiments similar to those described earlier for IVa and IVb.

The presence of the cyclopentenone moiety A in IVc was established as follows. Irradiation near 6·1 ppm (H_3) simplified the doublet of doublets at 7·75 (H_2) to a broad singlet by removing the larger (6·5 c/s) of the two couplings. The smaller coupling (2 c/s of H_2, 3 of H_3) was shown to be due to vicinal coupling with an allylic proton (H_1) near 3·1 ppm because irradiation in the latter region sharpened H_2 and H_3 into clean doublets (J = 6·5 c/s).

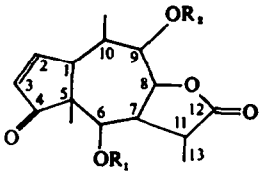
The doublet at 5·74 ppm (J = 8 c/s) assigned to H_6 was shown to be coupled to an allylic proton when irradiation near 3·1 ppm collapsed the doublet to a singlet. Since this proton must be attached to a carbon atom which is adjacent to a quaternary center, it cannot be placed at C_{10} adjacent to the allylic H_1. The other allylic proton must therefore be at C_7. Irradiation near 3·1 ppm also collapsed the doublets of H_{13a} and H_{13b}, thus confirming the allylic coupling between H_7 and H_{13a} and H_{13b},

[16] C. Djerassi, J. Osiecki and W. Herz, *J. Org. Chem.* **22**, 1361 (1957).
[17] W. Herz, A. Romo de Vivar, J. Romo and N. Viswanathan, *Tetrahedron* **19**, 1359 (1963).
[18] D. Rogers and Mazhar-ul-Haque, *Proc. Chem. Soc.* 92 (1963).
[19] M. T. Emerson, C. N. Caughlan and W. Herz, *Tetrahedron Letters* 621 (1964).
[20] W. Herz, Y. Kishida and M. V. Lakshmikantham, *Tetrahedron* **20**, 1986 (1964).
[21] W. Herz and M. V. Lakshmikantham, *Tetrahedron* **21**, 1711 (1965).
[22] We are greatly indebted to Drs. Herman Ziffer and Lin Tsai of the National Institutes of Health for determining the ORD curves.

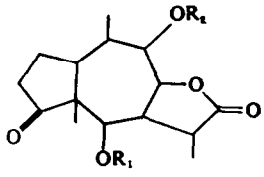
and caused simplification of the two proton multiplet at 4·8 ppm, indicating that H_8 is one of the protons in that region.

The signal due to the proton carrying the secondary methyl group was located near 2·1 ppm when irradiation in this region collapsed the methyl doublet to a singlet. This proton was shown to be coupled to a second allylic proton (which must be H_1 since H_7 had been shown to be flanked by protons giving rise to signals at 5·66 and 4·8 ppm) by irradiation at 2·1 which caused distinct perturbation in the multiplet near 3·1 ppm. This result placed the secondary methyl group unambiguously at C_{10}. Although the second acetate must therefore be placed at C_9, by exclusion, direct confirmation for structure IVc was obtained by the observation that irradiation of H_{10} (at 2·1) also simplified the multiplet near 5·0 ppm.

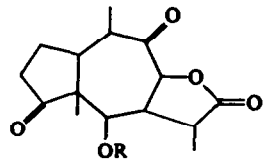
Since the chemical shifts and coupling constants in the NMR spectrum of IVc differ significantly from those observed in the fastigilin, helenalin and balduilin series and somewhat less so from those of mexicanin I,[23] but approximate those of bigelovin (VII)[21,24] extremely closely, we suggest for it the stereochemistry VIII. This is supported by the ORD curve which exhibits a negative Cotton effect.



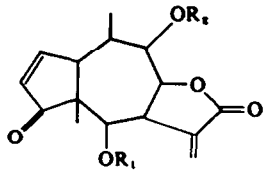
I a R_1 = angeloyl, R_2 = H
b R_1 = senecioyl, R_2 = H
c R_1 = senecioyl, R_2 = Ac



II a R_1 = $-C(=O)-CH(OCH_3)-CH_2CH_3$, R_2 = H
b R_1 = $-C(=O)-CH_2CH(CH_3)_2$, R_2 = H
c R_1 = $-C(=O)-CH_2CH(CH_3)_2$, R_2 = Ac
d R_1 = $-C(=O)-CH_2CH(CH_3)_2$, R_2 = Ms

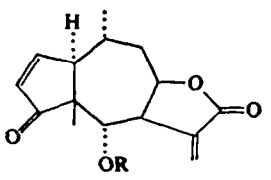


III a R = $-C(=O)-CH(CH_3)-CH_2CH_3$
b R = $-C(=O)-CH_2CH(CH_3)_2$



IV a R_1 = $-C(=O)-CH=C(CH_3)_2$, R_2 = H
b R_1 = $-C(=O)-CH=C(CH_3)_2$, R_2 = Ac
c R_1, R_2 = Ac

[23] E. Dominguez and J. Romo, *Tetrahedron* **19**, 1415 (1963).
[24] B. A. Parker and T. A. Geissman, *J. Org. Chem.* **27**, 4127 (1962).



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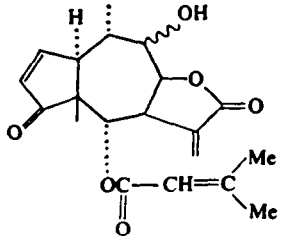

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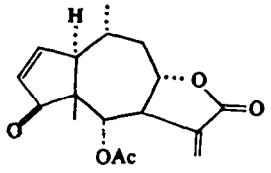

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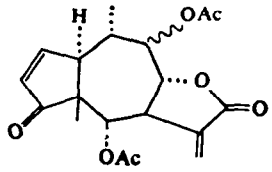

VIII

EXPERIMENTAL[25]

Extraction of Gaillardia Fastigiata *Greene*

(A) Leaves, flowers and small stems of *G. fastigiata* Greene, collected by Dr. H. F. L. Rock in Tarrant County, Texas during the summer of 1959 (Rock No. 1067), wt. 1020 g, were extracted with $CHCl_3$ for 2 days. The extract was concentrated at red. press, the residue taken up in 270 ml hot EtOH and diluted with 350 ml hot water containing 15 g lead acetate and 2 ml AcOH. After 2 days at room temp, the mixture was filtered, the filtrate concentrated to small volume at red. press. and extracted thoroughly with $CHCl_3$. The dried $CHCl_3$ solution was concentrated and the gummy residue, wt. 18 g, chromatographed over 220 g alumina (Alcoa F-20), solvent and eluent benzene $CHCl_3$ (1:1) and $CHCl_3$ (150 ml fractions). Fractions 7 and 8 (benzene–$CHCl_3$) and 9–14 ($CHCl_3$) gave a solid residue, total wt. 3·2 g. Fractions 15–17 ($CHCl_3$) and 18–25 ($CHCl_3$–MeOH 49:1 and 19:1) did not crystallize.

The solid was separated into benzene-soluble (fastigilin A) and benzene-insoluble (fastigilin B) material. Repeated recrystallizations of the benzene-soluble material from acetone–pet. ether, hot water and acetone–pet. ether furnished 0·5 g fastigilin A, m.p. 175–177°, $[\alpha]_D^{25}$ −81·6° (*c*, 0·5) λ_{max} 222 (ε = 18000) and 305 mμ, IR bands (KBr pellet) at 3500 (—OH), 1775 (γ-lactone) and 1715 cm^{-1} (conjugated ester and cyclopentenone), in $CHCl_3$ at 3600, 1778, 1722 (double intensity), 1650 (conjugated double bond) and 1590 cm^{-1}. (cyclopentenone double bond). Chromatography over acid-washed alumina failed to change the physical constants. (Found: C, 66·08; H, 7·08; O, 26·25. Calc. for $C_{30}H_{36}O_6$: C, 66·28; H. 7·23; O, 26·49%.)

Repeated recrystallization of the benzene-insoluble material from acetone–pet. ether furnished 0·8 g fastigilin B, m.p. 259–261°, λ_{max} 222·5 (ε = 22200) IR bands (KBr) 3500 (·OH), 1752 (γ-lactone), 1720 (unsaturated ester), 1705 (cyclopentenone) and 1650 cm^{-1} (conjugated double bond), in $CHCl_3$ at 1776, 1720 (double intensity), 1650 and 1585 cm^{-1}. The substance was too insoluble in the common solvents used for this purpose to permit reliable measurement of rotation. (Found: C, 66·45; H, 7·27; O, 26·42. Calc. for $C_{30}H_{36}O_6$: C, 66·28; H, 7·23; O, 26·49%.)

(B) *G. fastigiata*, above ground parts collected in western Oklahoma in June 1962 by Dr. C. S. Wallis, wt. 78 lbs, was ground and extracted in two batches of 36 and 42 lbs with $CHCl_3$. Workup in the usual manner yielded 240 and 255 g of crude gum which was chromatographed over silicic acid in several portions. The results of one chromatogram of 80 g crude gum from the first batch over

[25] M.ps are uncorrected. Analyses by Dr. F. Pascher, Bonn, Germany. UV spectra were run in 95% EtOH, IR spectra in $CHCl_3$ solution, rotations in $CHCl_3$, NMR spectra in $CDCl_3$ unless otherwise specified. Petroleum ether boiled at 30–60°. ORD curves were determined in MeOH solution by Drs. Herman Ziffer and Lin Tsai to whom we express our thanks.

1·3 kg silicic acid (Mallinckrodt 100 mesh) in benzene–$CHCl_3$ (1:1) are described. The column was successively eluted with 8 × 500 ml. of benzene–$CHCl_3$ (1:2), 8 × 500 ml. of benzene–$CHCl_3$ (1:3), 32 × 500 ml of $CHCl_3$, 17 × 500 ml $CHCl_3$–MeOH (99:1), 14 × 500 ml $CHCl_3$–MeOH (49:1), 6 × 500 ml. of $CHCl_3$–MeOH (97:3) and 17 × 500 ml $CHCl_3$–MeOH (19:1) and 4 × 500 ml $CHCl_3$–MeOH (9:1). The composition of each fraction was monitored by TLC. Fractions 1–28 gave gums which were complex mixtures, fractions 29–35 gave gums which exhibited similar TLC behavior (no indication of the presence of fastigilin A) but could not be induced to crystallize on rechromatography, fractions 38–49 gave a gum containing fastigilin C, wt. 3·6 g, which solidified on trituration with ether and was purified by rechromatography over acid-washed alumina (*vide infra*). Fractions 56–63 gave a gum (2·9 g) containing fastigilin B which solidified on trituration with either and was purified by rechromatography over alumina. Fractions 73–79 solidified on trituration with petroleum ether. Recrystallization from MeOH furnished hispidulin, m.p. 291–292°, which did not depress the m.p. of authentic material, UV, IR and TLC behavior identical with that of authentic material, triacetate identical with that of authentic material.

Crude fastigilin C was dissolved in $CHCl_3$ and rechromatographed over acid-washed alumina. The solid fractions were combined and recrystallized from acetone–isopropyl ether, m.p. 197–199°, $[\alpha]_D^{28}$ −85·8° (*c*, 1·11) λ_{max} 222·5 mμ (ε = 29300), IR bands (KBr) at 3600 (—OH), 1760 (γ-lactone), 1720 (conjugated ester), 1705 (cyclopentenone), 1655, 1645 and 1585 cm^{-1} (conjugated double bonds), ORD curve (*c*, 0·632) $[\alpha]_{500}$ −436°, $[\alpha]_{351}$ −1590°, $[\alpha]_{308}$ −98°, $[\alpha]_{341}$ −6330, $[\alpha]_{240}$ −6260 (last reading). (Found: C, 66·33, H, 6·71; O, 26·64. Calc. for $C_{20}H_{24}O_6$: C, 66·65; H, 6·71; O, 26·64%.)

Chromatography of the crude gum from the second batch of *G. fastigiata* yielded very little fastigilin C followed by mikanolide, $C_{15}H_{14}O_6$, m.p. 250° (dec), and then by fastigilin B and hispidulin. Total yields from both batches were fastigilin B 0·75 g, fastigilin C 1·8 g, $C_{15}H_{16}O_6$ 2·5 g and hispidulin 0·4 g.

Tetrahydrofastigilin A (IIa). A solution of 0·25 g fastigilin A in 50 ml EtOH was hydrogenated for 4 hr at a pressure of 45 lbs/in² in the presence of 50 mg. of 10% Pd–C. The filtrate was evaporated at red. press. and the residue recrystallized from benzene–pet. ether, yield 0·2 g, m.p. 118–120° (Found: C, 65·43; H, 8·05; O, 26·17. Calc. for $C_{20}H_{30}O_6$: C, 65·55; H, 8·25, O, 26·20.%)

Dehydrotetrahydrofastigilin A (IIIa). A solution of 0·175 g of the above in 3 ml ACOH was mixed with 0·175 g chromic acid in 3 ml ACOH and a few drops of water and allowed to stand overnight. Excess oxidizing agent was destroyed with MeOH, the solution evaporated *in vacuo*, the residue digested with water and extracted with $CHCl_3$. The extract was washed, dried and evaporated and the residue recrystallized from ether–pet. ether, yield 0·05 g, m.p. 120–122°, IR bands (KBr) at 1795 (γ-lactone), 1745 (ester and cyclopentanone) and 1710 $cm.^{-1}$ (cycloheptanone). The IR spectrum was almost, but not quite, superimposable on that of IIIb, mixed m.p. with IIIb 116–118°. (Found: C, 66·10; H, 7·80; O, 26·36. Calc. for $C_{20}H_{28}O_6$: C, 65·91; H, 7·74; O, 26·34%.)

Acetylfastigilin B (Ic). A mixture of 0·2 g fastigilin B, 1 ml pyridine and 2 ml Ac_2O was warmed on the steam bath until solution was complete, allowed to stand overnight at room temp, poured on ice, filtered, washed with water, dried and recrystallized from AcOEt–pet ether. The acetate, wt. 0·165 g, melted at 260–262° (dec) $[\alpha]_D^{28}$ −14° (*c*, 1·00), mixed m.p. with starting material about 230°, infrared bands at 1775 (γ-lactone), 1742 (acetate), 1717 (unsaturated ester and cyclopentenone), and 1650 cm^{-1}. (Found: C, 65·48; H, 7·07; O, 27·58. Calc. for $C_{22}H_{28}O_7$: C, 65·33; H, 6·98; O 27·69%.)

Tetrahydrofastigilin B (IIb). A solution of 0·3 g Ib in 50 ml EtOH was reduced at 45 lbs/in² with 50 mg 10% PdC. The solution was filtered, evaporated at red. press. and the residue recrystallized from acetone–pet. ether, yield 0·2 g, m.p. 170–172°, $[\alpha]^{300}$ +115° (*c*, 1·00), IR bands (KBr) at 3500 (—OH), 1760 (γ-lactone) and 1737 cm^{-1} (ester and cyclopentanone) in chloroform at 3600, 1765 and 1740 cm^{-1}, ORD curve (*c*, 0·0432) $[\alpha]_{400}$ −153°, $[\alpha]_{316}$ +970°, $[\alpha]_{310}$ +855° (shoulder), $[\alpha]_{277}$ −1210°, $[\alpha]_{221}$ −318°, $[\alpha]_{220}$ −520° (last reading). (Found: C, 65·54; H, 8·25; O, 26·16. Calc. for $C_{20}H_{30}O_6$: C, 65·55; H, 8·25; O, 26·20%.)

Acetyltetrahydrofastigilin B (IIc). Acetylation of 35 mg of the above in the usual manner furnished, after recrystallization from AcOEt–pet. ether, 20 mg of the acetate, m.p. 168–170°, IR bands (KBr) at 1787 (γ-lactone), 1742 and 1725 cm^{-1} (ester, cyclopentanone and acetate). (Found: C, 64·64; H, 7·94, O, 27·32. Calc. for $C_{22}H_{32}O_7$: C, 64·68; H, 7·90; O, 27·42%.)

Dehydrotetrahydrofastigilin B (IIIb). Oxidation of 0·15 g IIb in 3 ml AcOH with 0·15 g chromic acid in the manner described for IIa furnished, after chromatography over acid-washed alumina and

elution with benzene–ether (3:1) a gum which on leaving in aqueous MeOH for a few days crystallized in the form of silky needles, yield 50 mg, m.p. 112–114°. A second chromatographic purification raised the m.p. to 115–116°, IR bands (KBr) at 1782 (γ-lactone), 1745 (ester and cyclopentanone) and 1718 cm^{-1} (cycloheptanone), in $CHCl_3$ 1782, 1745 and 1718 cm^{-1}. (Found: C, 66·54; H, 8·15. Calc. for $C_{30}H_{38}O_6$: C, 66·91; H, 7·74%.)

Mesylate of IIb(IId). A solution of 0·325 g IIb in 2·5 ml pyridine was chilled and mixed with 0·5 ml methanesulfonyl chloride. After one night in the refrigerator the mixture was poured on ice, filtered, washed with water, dried and recrystallized from acetone–pet ether, wt. 0·15 g, m.p. 173–174°, IR bands (KBr) at 1780 and 1745 cm^{-1} (double strength). (Found: C, 56·40; H, 7·18; S, 7·08. Calc. for $C_{21}H_{32}O_8S$: C, 56·74; H, 7·26; S, 7·20%.)

In an attempt to correlate the fastigilins with other pseudoguaianolides, IId was refluxed with collidine, lutidine and other bases. This resulted in recovery of IId.

Reactions of Fastigilin C. (A) A solution of 0·114 g IVa in 30 ml $CHCl_3$ was ozonized at 0° for 1 hr. The mixture was steam distilled into 50 ml ice-cold dimedone solution which was concentrated at red. press. to remove organic solvents. Upon cooling there was obtained 14 mg of the dimedone derivative of formaldehyde, undepressed on admixture of an authentic sample.

A solution of 0·104 g IVa in 25 ml $CHCl_3$ was ozonized at 0° for 1 hr. The mixture was steam-distilled into an ethanolic solution of 2,4-dinitrophenylhydrazine hydrochloride. This was refluxed for 20 min, and evaporated *in vacuo*. The residue was extracted with $CHCl_3$, the extract washed, dried and evaporated and the residue chromatographed over silicic acid. Benzene–$CHCl_3$ (1:1) eluted acetone dinitrophenylhydrazone, m.p. 124–126°, identified by comparison (mixed m.p., IR, TLC) with an authentic sample.

(B) Acetylation of 0·2 g IVa in the usual manner gave 0·12 g solid material (IVb) m.p. 150° (with prior softening) which could not be recrystallized satisfactorily even after chromatography, IR bands at 1770 (γ-lactone), 1746 (acetate), 1725 (ester and cyclopentenone), 1650 and 1585 cm^{-1} (double bonds), single spot on TLC. The NMR spectrum also indicated its homogeneity.

Catalytic reduction of 0·4 g IVa in 50 ml EtOH with 10% Pd–$CaCO_3$ at 32 lbs/in^2 and work-up in the usual manner furnished 0·3 g IIb, m.p. 169–171°, IR spectrum superimposable on that of authentic material. For further identification, the reduction product was converted to IIc, IR spectra superimposable, mixed m.p. undepressed.

Compound VIII from G. pinnatifida *Torr.* Isolation and properties of this substance have been described previously. ORD curve (*c*, 0·064) $[\alpha]_{500}$ +20°, $[\alpha]_{360-365}$ −580° (shoulder), $[\alpha]_{352.5-355}$ −656°, $[\alpha]_{335}$ +6900°, $[\alpha]_{320}$ −8600° (last reading).