1785 (s), 1680 (m), 1660 (s), 1600 cm^{-1}. The product was not characterized further.

Registry No.—**1,** 55871-81-3; **2,** 19656-65-6; **3,** 55871-82-4; **4,** 55871-83-5; **6a,** 55871-84-6; **6b,** 55871-85-7; **6c,** 55871-86-8; **7a,** 691-24-7; **7b,** 622-16-2; **7c,** 14041-89-5; **8a,** 42136-40-3; **8b,** 34362-08-8; **8c,** 55871-87-9; **9a,** 55871-88-0; **9b,** 55871-89-1; **9c,** 55871-90-4; **10,** 22975-87-7; **11,** 55871-91-5; **12,** 43023-11-6; **13a,** 55871-92-6; **13b,** 55871-93-7; **14,** 19656-62-3; **15a,** 55871-94-8; **16a,** 55871-95-9; **16b,** 55871-96-0; **17a,** 55871-97-1; **17b,** 55871-98-2; **18a,** 55871-99-3; 1-carboethoxy-1-phenyl-4-isopropylsemicarbazide, 55872-00-9.

References and Notes

(1) Supported in part by the National Science Foundation.
(2) F. D. Greene and J. F. Pazos, *J. Org. Chem.*, **34,** 2269 (1969).
(3) G. Zinner and H. Gross, *Chem. Ber.*, **105,** 1709 (1972).
(4) (a) K. Hartke and E. Palov, *Chem. Ber.*, **99,** 3155 (1966); (b) Farbenfabriken Bayer AG, German Patent 1,131,661 (1962); *Chem. Abstr.*, **58,** 1401e (1963); (c) H. Ulrich and A. A. R. Sayigh, *J. Org. Chem.*, **28,** 1427 (1963).
(5) G. Voss, E. Fischer, and H. Werchan., *Z. Chem.*, **13,** 102 (1973).
(6) H. Quast and E. Schmitt, *Chem. Ber.*, **103,** 1234 (1970).
(7) A. M. Simonov and A. F. Pozharskii, *Zh. Obshch. Khim.*, **33,** 2350 (1963).
(8) Plausible routes may be found for the conversion of structures of types C and E to F by *alkoxide*, but we know of no precedent for the conversion of compounds such as C and E to species containing a nitrogen–nitrogen bond by hydroxide ion.
(9) For related small-ring isomerizations, see ref 6 and H. Quast and W. Risler, *Angew. Chem., Int. Ed. Engl.*, **12,** 414 (1973); R. L. Camp and F. D. Greene, *J. Am. Chem. Soc.*, **90,** 7349 (1968).
(10) R. Huisgen, R. Grashey, and J. Sauer in "The Chemistry of the Alkenes", S. Patai, Ed., Interscience, New York, N.Y., 1964, Chapter 11; E. Schmitz, "Dreiringe mit Zwei Heteroatomen", Springer-Verlag, West Berlin, 1967.
(11) K. Hunger, *Tetrahedron Lett.*, 5929 (1966).
(12) I. G. Hinton and R. F. Webb, *J. Chem. Soc.*, 5051 (1961).
(13) (a) T. W. Campbell, J. J. Monagle, and V. S. Foldi, *J. Am. Chem. Soc.*, **84,** 3673 (1962); (b) H. C. A. van Beek and P. M. Heertjes, *Melliand Textilber*, **44,** 987 (1963).
(14) R. Stolle, *Chem. Ber.*, **32,** 2238 (1899).
(15) W. D. Emmons, *J. Am. Chem. Soc.*, **79,** 5739 (1957).
(16) N. A. Porter and L. J. Marnett, *J. Am. Chem. Soc.*, **95,** 4361 (1973). We wish to thank N. A. Porter for a sample.
(17) C. P. Pacilly, *Recl. Trav. Chim. Phys-Bas*, **55,** 101 (1936).

A *trans*-1,2-*cis*-4,5-Germacradienolide and Other New Germacranolides from *Tithonia* Species[1]

Werner Herz* and Ram P. Sharma

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

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Isolation and structure determination of two new germacranolides, tifruticin (**1a**) and deoxyfruticin (**4a**), from *Tithonia fruticosa* Canby and Rose are described. Deoxyfruticin is the first naturally occurring *trans*-1,2-*cis*-4,5-germacradienolide. Structures were determined by chemical transformations and extensive use of ^1H and ^{13}C NMR spectrometry. Structures are suggested for tirotundin and its ethyl ether, two new germacranolides from *Tithonia rotundifolia* (Mill.) Blake.

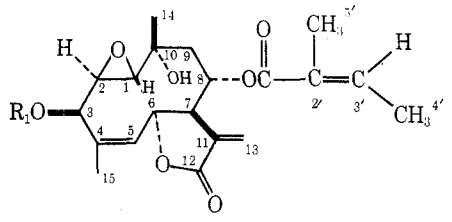
As part of our search for secondary metabolites of Compositae with potential biological activity, we have examined collections of *Tithonia fruticosa* Canby and Rose and *Tithonia rotundifolia* (Mill.) Blake (Heliantheae, subtribe Helianthinae). The former yielded two closely related new germacranolides, tifruticin (**1a**) and deoxyfruticin (**4a**). Although only small amounts of these compounds were available, the complete structure and stereochemistry has been elucidated. *T. rotundifolia* afforded the new germacranolide tirotundin and its ethyl ether, for which structures **9a** and **9b** are suggested in preference to **10a** and **10b.**

Trifruticin (**1a**), mp 141°, $C_{20}H_{26}O_7$ (mass spectrum and elemental analysis), $[\alpha]^{22}D$ −22°, was a conjugated γ-lactone (ir bands at 1760 and 1640 cm^{-1}, strong uv end absorption) and had at least one hydroxyl group (ir band at 3400 cm^{-1}). In the ^1H NMR spectrum of **1a,** the proton under the (secondary) hydroxyl group was located at 4.46 ppm by D_2O exchange and by its paramagnetic shift to 5.33 ppm on acetylation of tifruticin to **1b.** In the 270-MHz NMR spectrum of the latter compound, all signals were well separated; hence decoupling experiments on **1b** afforded the full structure of tifruticin.

The NMR spectrum of **1b** (Table I) exhibited the typical two doublets of H_a and H_b in partial structure A at 6.38 and 5.92 ppm. Spin decoupling experiments involving H_a and H_b established the location of the H_c multiplet at 3.22 ppm. Irradiation at the frequency of H_c converted a doublet of doublets at 5.05 ppm to a doublet (J = 10 Hz) and a multiplet at 5.21 ppm was also simplified. Thus H_d and H_e are at 5.05 and 5.21 ppm, respectively, or the reverse. If it be assumed provisionally that the signal at higher field is H_d, as is generally the case, the signal at lower field could be assigned tentatively to a proton on a carbon carrying a conjugated ester function whose presence was indicated by an ir band at 1700 cm^{-1}.

Since the low-resolution mass spectrum of tifruticin displayed diagnostically important peaks at *m/e* 278 (M^+ − 100), 260 (M − 100 − 18), and 83 (base peak), the inference was drawn that a five-carbon ester side chain was present. The nature of the ester (partial structure B) was revealed by the NMR spectrum, which had a vinyl multiplet at 6.20 ppm coupled to a three-proton multiplet at 2.01 ppm and another methyl multiplet at 1.88 ppm, all characteristic of an angeloyl group.

Irradiation at the frequency of H_e (5.21 ppm) affected the H_c multiplet, collapsed a doublet of doublets at 2.22 ppm to a doublet (J = 15 Hz), and affected a partially obscured one-proton signal near 2.00 ppm. Irradiation near 2 ppm collapsed the doublet of doublets at 2.22 ppm to a doublet (J = 6 Hz) and converted the 5.21-ppm multiplet to a triplet, thus demonstrating that H_e was adjacent to a methylene group (H_f). Irradiation at the frequency of H_c (5.05 ppm) collapsed a broadened doublet at 5.52 ppm to a broad singlet. The broadening of this signal (H_g) could be traced to a small coupling with a narrowly split three-proton multiplet at 2.10 ppm. Thus partial structure A could be extended to C, where the symbol ■ represents quaternary carbon.



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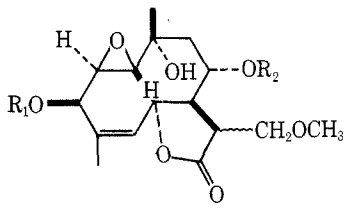
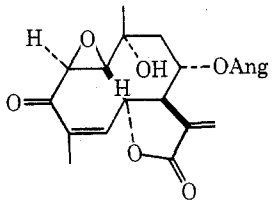
b, R = Ac



2a, R_1, R_2 = H
b, R_1, R_1 = Ac



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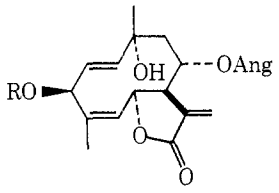
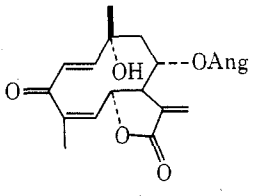


4a, R = H
b, R = Ac



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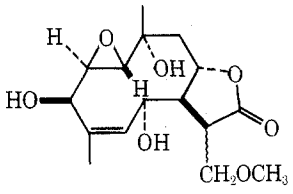

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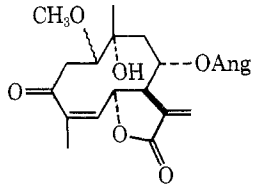
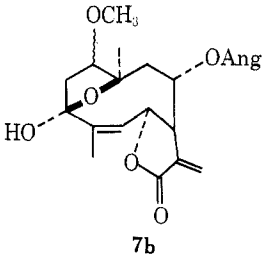
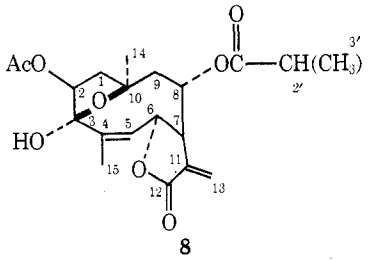


7a



7b



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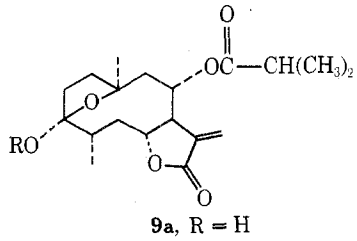
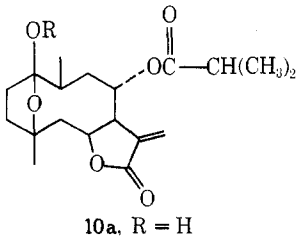


9a, R = H
b, R = Et



10a, R = H
b, R = Et

It was further shown that a somewhat broadened doublet at 3.36 ppm (H_i, J = 2.5 Hz) and a sharp doublet at 3.15 ppm (H_j) constituted an AB system, the broadening of H_i being due to coupling with the proton under the acetate at 5.33 ppm (H_h, vide supra). The chemical shift of the AB system suggested that it represented two protons in an epoxide ring which is included in partial structure D.

Six of the seven oxygen atoms and 20 of the 22 carbon atoms of tifruticin were accounted for by C and D. The remaining two carbons and one oxygen had to be assigned to the grouping CH_3COH where the hydroxyl is tertiary, since the ir spectrum of **1b** still exhibited hydroxyl absorption and the NMR spectra of **1a** and **1b** displayed a three-proton singlet at 1.39 ppm typical of methyl on carbon attached to oxygen.

All of the above information was accommodated by formulas **1a** (devoid of stereochemistry) or E with the proviso mentioned earlier that the proton under the lactone oxygen (H_d) is represented by the signal at 5.05 ppm, i.e., that the lactone ring is closed to C-6. This was confirmed as follows.

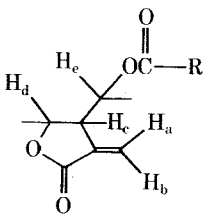
Methanolysis of **1a** (NaOMe, MeOH) gave **2a** by loss of the ester side chain and addition of the elements of methanol to the α,β-unsaturated lactone. The proton under the newly freed hydroxyl function (H_e) now appeared as a multiplet at 3.94 ppm (Table I), and was further identified by its paramagnetic shift on acetylation to **2b.** Decoupling experiments on **2a** confirmed that the H_d signal had remained at 5.03 ppm and was coupled to a vinyl proton at 5.44 ppm, whereas the 3.94-ppm signal was coupled to a methylene group. Hence the lactone ring of tifruticin is closed to C-6.

That the secondary hydroxyl group of tifruticin was allylic and that formula E must be rejected was established by MnO_2 oxidation of **1a** to the α,β-unsaturated ketone **3** [double-strength ir band at 1700 cm^{-1}, uv λ_{max} 235 nm (ϵ 9000)] in whose NMR spectrum (Table I) the H-2 signal was shifted downfield to 4.07 ppm. Finally the ^{13}C NMR spectrum of **1a** (Table II) was fully consonant with the assigned structure **1a.**

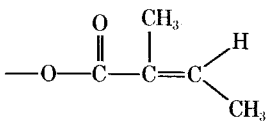
Before discussing the stereochemistry of tifruticin, mention should be made of deoxytifruticin (**4a**), which was isolated from *T. fruticosa* in very low yield and whose purification was attended with considerable difficulties (see Experimental Section). The NMR spectrum of **4a** (Table I) resembled that of **1a** with the exception that the AB system of H-1 and H-2 was displaced downfield by 2.5 ppm, an observation which, taken together with the empirical formula $C_{20}H_{26}O_6$, suggested that the epoxide ring of **1a** had been replaced by a double bond. This was in complete harmony with the ^{13}C NMR spectrum (Table II) and could be confirmed by peracid oxidation of **4a** to **1a,** and of **4b** to **1b.**

MnO_2 oxidation of **4a** afforded the cross conjugated dienone **5.** The ir spectrum of this substance exhibited a very strong band at 1650 cm^{-1} attributable to the new chromophore. The uv spectrum showed the expected maximum at 250 nm (ϵ 8500), while in the NMR spectrum of **5** the resonances of H-1 and H-2 had moved still further downfield, in agreement with the postulated structure.

As regards the stereochemistry of **1a** and **4a,** if the usual assumption be made that the C-7 side chain is β oriented as in all sesquiterpene lactones of authenticated absolute stereochemistry, the value of $J_{6,7}$ (10 Hz) requires that H-6 and H-7 have a trans relationship, i.e., that the lactone ring by trans fused and H-6 be β. Furthermore, NaOH hydrolysis of **2a** followed by acidification resulted in isolation of a product **6** with a reorientated lactone ring. Although shortage of material prevented adequate characterization, the NMR spectrum exhibited the H-6 and H-8 signals near 4.5 ppm and the H-5 signal at 5.00 ppm. This suggested that



A



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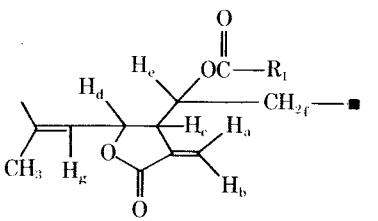
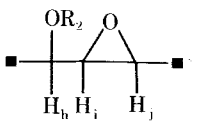
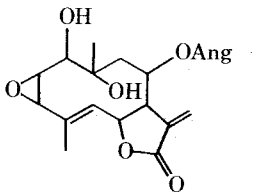


C



D



E

Table I
^{1}H NMR Spectra of Compounds from Tithonia[a]

Compd	H-1	H-2	H-3	H-5	H-6	H-7	H-8	H-9	H-13	H-14[b]	H-15[b]	H-3'	H-4'[b]	H-5'[b]	Misc
1a	3.30[c]	3.30[c]	4.46 br	5.55 dd (10, 1.5)	5.19 dd (10, 10)	3.22 m (10, 3.3, 3.1, 4)	5.19 m	2.15 dd (15, 6) *e*	6.35 d (3.3) 5.88 d (3.1)	1.38	2.01 d (1.5)	6.17 m (7, 1.5)	1.96 m (7, 1.5)	1.88	
1b	3.15 d (2.5)	3.36 d br (2.5)	5.33 br	5.52 d br (10, 1.5)	5.05 dd (10, 10)	3.22 m (10, 3.3, 3.1, 4)	5.21 m	2.22 dd (15, 6) *e*	6.38 d (3.3) 5.92 d (3.1)	1.39	2.10 d (1.5)	6.20 m (7, 1.5)	2.01 m (7, 1.5)	1.88 m	2.04 (Ac)
2a	*e*	*e*	4.44 br	5.44 d br (10, 1.5)	5.03 dd (10, 10)	2.38 m	3.94 m	*e*	*e*	1.42	1.96 d (1.5)				3.44 (OMe)
2b	3.16 d (2.5)	3.33 d br (2.5)	5.32 br	5.44 d br (10, 1.5)	4.92 dd (10, 10)	*e*	5.14 m	*e*	3.62 dd (10, 3) 3.86 dd (10, 2)	1.43	2.07 d (1.5)				3.33 (OMe) 2.04, 2.04 (Ac)
3	3.02 d (3.0)	4.07 d (3.0)		5.74 d br (10, 1.5)	4.54 dd (10, 10)	3.38 m (10, 4, 3.3, 3.1)	5.47 m	2.16[c]	6.38 d (3.3) 5.98 d (3.1)	1.42	20.6 d (1.5)	6.20 m (7, 1.5)	2.01 m (7, 1.5)	1.89 m	
4a	5.77 d (17)	6.08 dd (17, 1.5)	4.69 br (1.5)	5.27 d br (10, 1.5)	5.77 dd (10, 10)	3.05 m (10, 3.3, 3.0, 4)	5.18 m	2.14 dd (15, 6) *e*	6.34 d (3.3) 5.82 d (3.0)	1.38	1.97 d (1.5)	6.22 m (7, 1.5)	2.02 m (7, 1.5)	1.87 m	
4b	5.60 d (17)	6.07 dd (17, 1.5)	5.48 br (1.5)	5.25 d br (10, 1.5)	5.57 dd (10, 10)	3.05 m (10, 4, 3.3, 3.0)	5.18 m	2.19 dd (15, 6) *e*	6.35 d (3.3) 5.82 d (3.0)	1.37	2.02 br (1)	6.22 m (7, 1)	2.02 m (7, 1)	1.87 br	2.13 (Ac)
5	6.49 d (17)	6.25 d (17)		5.87 d br (9, 1.5)	5.40 d br[f] (9)	3.55 m[f]	5.40 m	2.53 dd (15, 6)	6.36 d (3.3) 5.82 d (3.0)	1.53	1.95 d (1.5)	6.08 m (7, 1.5)	1.92 m (7, 1.5)	1.75 m	

7	4.02 dd (10, 6)	2.60 dd (14, 6)		5.64 d br (5, 1)	5.72 dd (5, 5)	4.13 m	5.42 m	*e*	6.26 d (2.1) 5.60 d (2.0)	1.54	1.82 br	6.05 m	1.90 m	1.73 br	3.39 (OMe)
8[f]	*e*	5.36 d br	*e*	5.60 d[g]	5.50 m	4.06 m	5.50 m		6.23 d (2.4) 5.60 d (2.0)	1.50	1.77 t (1.5)	1.05 d (7)	1.07 d (7)		2.12 (Ac)
9a	*e*	*e*	*e*	*e*	4.57 dd br (7, 10.5, 1)	4.11 m (7, 3.4, 3.0, 1.5)	5.54 m	*e*	6.25 d (3.4) 5.54 d (3.0)	1.45	1.13 d (7)	1.05 d (7)	1.08 d (7)		
9b	*e*	*e*	*e*	*e*	4.50 dd br (7, 10.5, 1)	4.05 m (7, 3.4, 3.0, 1.5)	5.55 m	*e*	6.24 d (3.4) 5.53 d (3.0)	1.42	1.04 d (7)	1.02 d (7)	1.02 d (7)	1.12 t (7)[b] 8.33 m 3.50 m	

[a] Run in $CDCl_3$ at 270 MHz on a Bruker HFX-270 instrument with Me_4Si as internal standard. Values are in parts per million; d, doublet; t, triplet; br, broadened singlet; m, multiplet. Unmarked signals are singlets. Figures in parentheses are coupling constants in hertz. [b] Intensity three protons. [c] Intensity two protons. [d,d] Center of AB system. [e] Signal in methylene envelope or obscured. [f] $J_{6,7} < 1$; $J_{7,8} < 1$. [g] From ref 4, run at 90 MHz.

Table II
^{13}C NMR Spectra of Tifruticin and Congeners[a]

Signal no.	1a	4a	Assignment[b]	9a	Assignment[b]
1	169.3 s	169.4 s	C-1′	176.1 s	C-1′
2	167.3 s	168.0 s	C-12	169.4	C-12
3	143.8 s	146.8 s	C-4	137.2 s	C-11
4	140.1 d	140.7 d	C-3′	121.4 t	C-13
5	134.9 s	135.5 s	C-11	108.8 s	C-3
6	127.1 s	127.1 s	C-2′	81.3 d	C-6
7	126.8 d	125.9 d	C-5	80.0 s	C-10
8	122.9 t	122.6 t	C-13	69.8 d	C-8
9	74.4 d	73.9 d[c]	C-6	47.9 d	C-7
10	69.9 d[c]	72.9 d[c]	C-3	43.4 d	C-4
11	67.4 s	70.8 s	C-10	42.2 t[c]	C-9
12	67.1 d[c]	68.9 d	C-8	38.9 t[c]	C-1
13	60.0 d[d]	131.9 d[d]	C-1	38.4 t[c]	C-2
14	56.9 d[d]	130.5 d[d]	C-2	38.0 t[c]	C-5
15	51.2 d	51.3 d	C-7	34.1 d	C-2′
16	41.3 t	43.8 t	C-9	26.9 q	C-14
17	27.8 q	29.5 q	C-14	19.1	C-15
18	25.4 q	25.1 q	C-15	18.7	C-3′
19	20.5 q	20.4 q	C-4′	18.6	C-4
20	15.9 q	15.9	C-5′		

[a] Run in $CDCl_3$ on Bruker HFX-270 instrument. [b] Tentative assignments based on predicted shifts, comparisons with data in the literature (for references see W. Herz, I. Wahlberg, C. S. Stevens, and P. S. Kalyanaraman, *Phytochemistry*, in press) and spectra of lactones of known structure in our files. [c,d] Probable assignments, may be interchanged.

the C-8 side chain of **1a** and **4a** was α oriented (for further evidence on this point, vide infra), since germacranolides containing lactonizable α-oxygen groups at C-6 and C-8 preferably lactonize toward C-8.[2]

The small paramagnetic shift (0.2 ppm) of the H-5 signal accompanying the oxidation of **1a** to **3** was noteworthy and could be explained most satisfactorily by assuming that the carbonyl group at C-3 was twisted somewhat out of the plane of the C-4, C-5 double bond, a situation which could arise only if the double bond were cis.[3] The correctness of this deduction was demonstrated by the existence in **1a** of a nuclear Overhauser effect between H-15 and H-5. Irradiation at the frequency of the methyl group attached to C-4 produced a 12.5% enhancement in the integrated intensity of the H-5 signal.

The 1,2 double bond of **4a** must be trans because of the high value of $J_{1,2}$ (15 Hz); consequently deoxytifruticin represents the first example of a *trans*-1,2-*cis*-4,5-germacradienolide. Since epoxidation with *m*-chloroperbenzoic acid is known to proceed stereospecifically, H-1 and H-2 of **1a** are also trans. Now H-1 and H-6 of **3** are shifted upfield relative to H-1 and H-6 of **1a** and **1b** (Table I) presumably because these protons are located within the shielding cone of the new ketone group. Since H-6 is β, H-1 must be β also and H-2 is α. The small value of $J_{2,3}$ (<1 Hz) further indicates that the dihedral angle between H-2 and H-3 of **1a** and **1b** is close to 90°, in which case H-3 must be α oriented (models).

The chance observation that the uv absorption of **5** decreased on standing in methanol solution offered not only a clue to the stereochemistry at the remaining center C-10, but also provided additional evidence for the previous conclusions about the stereochemistry of tifruticin. That the product (**7**) of this transformation had been formed by addition of the elements of methanol to the conjugated C-1, C-2 double bond was indicated by its mass spectrum, the presence of a methoxyl signal in the NMR spectrum, and the upfield shifts of H-1 and H-2 (Table I). However, the ir

band at 1700 cm^{-1} was relatively weak compared with the analogous band of **3** which represents the combined cyclopentenone–conjugated ester chromophore. Consequently we assumed that **7** was predominantly in the hemiketal form **7b**, a surmise which was strengthened by comparison of the NMR spectrum of **7** with that of woodhousin (**8**,[4] Table I). In fact, since the chemical shifts of H-5, H-6, H-7, H-8, H-13, H-14, and H-15 and the coupling constants involving H-5, H-6, H-7, and H-8 were so similar, it was concluded that the stereochemistry of **7**, and hence that of **1a**, at C-5, C-6, C-7, C-8, and C-10 was the same as that of woodhousin.

We have commented previously[5] on the unusual low-field shift of the H-7 resonance (~4.1 ppm) in woodhousin and certain other cis C-4, C-5 germacranolides (erioflorin[4] and its congeners,[6] heliangin[7]) similar to **7**. In these compounds, H-7 is strongly deshielded by the oxygen atom attached to C-10.[8] The H-7 resonance of **7** is also strongly deshielded; models show that H-7 comes close to the acetal oxygen only if the absolute configuration of **7** at C-10 is *R* (if the absolute configuration of C-7 is as written) and the C-3 hydroxyl is α. Therefore the tertiary hydroxyl group on C-10 of tifruticin (**1a**) is α.[10]

The CD curve of **3** exhibits a negative Cotton effect, while that of **1a** is positive although no change has occurred in orientation of the lactone ring and stereochemistry at C-6. This reinforces our earlier conclusion[11] that the empirical rule relating the sign of the lactone Cotton effect to the type of lactone ring closure[12] is not generally applicable to *cis*-Δ^4-germacranolides. Similarly, $J_{7,13}$ for **7** is <3 in violation of Samek's rule,[13] as is true for other *cis*-Δ^4-germacranolides,[14] while $J_{7,13}$ for **1a, 1b, 3, 4a, 4b,** and **5** is >3. Obviously, the magnitude of $J_{7,13}$ depends on the conformation of the unsaturated germacranolide ring system and not on the stereochemistry of the lactone ring fusion per se.

The main sesquiterpene lactone constituent of *T. rotundifolia* was named tirotundin, $C_{19}H_{28}O_6$, mp 141°, $[\alpha]D$ −77°. The ^1H NMR spectrum (Table I) indicated the presence of partial structure A; this was confirmed by spin decoupling in the manner described for tifruticin, which also permitted identification of the H_d resonance as the more shielded of two signals in the ester region (4.57 vs. 5.54 ppm). Appropriate peaks at 1.05, 1.08 (two methyl doublets), and 2.44 ppm (septet) and fragmentation under electron impact (diagnostically important peaks at *m/e* 264, 247, and 71, the last base peak) showed that the ester side chain was isobutyrate.

The occurrence of the H_c multiplet at the same low frequency (4.11 ppm) as in **7** suggested that tirotundin might be a saturated (because of the analysis and the upfield shift of H_d) hemiketal of the woodhousin type, especially since the ir spectrum exhibited hydroxyl absorption and the NMR spectrum contained no signal indicative of a primary or secondary hydroxyl group. This deduction was supported by the ^{13}C NMR spectrum (Table II), which contained a singlet at 108.8 ppm, characteristic of tetravalent carbon carrying two oxygens, and another singlet at 80.0 ppm which must represent the carbon atom at the other terminus of the acetal linkage. The latter is also attached to a methyl group (^1H methyl singlet at 1.45, ^{13}C methyl quartet at 26.9 ppm).

The foregoing information leads to formulas **9a** (devoid of stereochemistry) or **10a**. Since the methylene signals of tirotundin were not sufficiently well separated at 270 MHz even in the presence of shift reagents to permit their unambiguous identification by double resonance, it was not possible to decide unequivocally between these two alternatives. However, irradiation at the frequency of H-6 did not appear to affect a partially obscured doublet of doublets, an observation which appears to favor the biogenetically more plausible **9a** over **10**. Moreover, the paramagnetic chemical shift of H-7 is highly characteristic of germacranolides containing an oxygen bridge linking C-3 and C-10 (vide supra); compounds of type **10** are so far unknown. Unfortunately, several attempts to distinguish between the two possibilities by chemical means failed.

The minor constituent of *T. rotundifolia* which had formula $C_{21}H_{32}O_6$ was easily recognized as the ethyl acetal of tirotundin **9b** or **10b** (mass spectrum, Tables I and II) possibly formed from tirotundin during the isolation process one stage of which employs ethanol or during the tedious chromatographic purification by reaction with trace amounts of ethanol in chloroform.

Since the chemical shifts of H-7 and H-8 are the same as those of the corresponding signals in the spectra of **7** and woodhousin, the C-8 ester side chain of tirotundin and its acetal is undoubtedly α oriented also. Because $J_{7,13a} > 3$ and the lactone Cotton effect is negative, the lactone ring is trans fused;[16] hence H-6 is β. Now if the gross structure of tirotundin is **9a,** inspection of the various models of **9a** with H-6 and H-8β and H-7α reveals that H-7 approaches the tetrahydrofuran oxygen only when the configuration at C-10 is *S* and C-3 OH is α (*R* configuration) as represented in the formula. Finally, the chemical shift of the C-10 methyl group suggests that it is cis to the hydroxyl at C-3, hence α oriented; this would also be the thermodynamically favored orientation in the ketol corresponding to **9a.**

Experimental Section

Experimental details have been specified previously.[16]

Extraction of *Tithonia fruticosa*. Above-ground parts of *T. fruticosa* Canby and Rose, wt 0.45 kg, collected by Mr. Juan Arguelles near Curahui, Sonora, Mexico in 1959 under USDA auspices (Arguelles No. 124 and 129, A. 5307 and A. 5308) was extracted with $CHCl_3$ and worked up in the usual fashion.[17] The crude gum, wt 6.0 g, was chromatographed over 200 g of silicic acid, 200-ml fractions being collected in the following order: 1–10 (Bz), 11–20 (Bz–$CHCl_3$, 10:1), 21–30 (Bz–$CHCl_3$, 1:1), 31–40 (Bz–$CHCl_3$, 1:10), 41–50 ($CHCl_3$), 51–60 ($CHCl_3$–MeOH, 20:1). Fractions 32–38 gave a mixture of two lactonic components which was separated into its constituents by preparative tlc on silica gel $PF_{255-366}$ (solvent hexane–ethyl acetate 3:2). The plate (20 × 40 cm, thickness 1 mm) was developed *six times;* after each development the plate was fully dried by leaving it in a hood for 1 hr. The two bands did not separate when the plate was developed only twice or three times. The upper band (**4a**) was obtained as a gum which could not be induced to crystallize, ir bands at 3400, 1760, 1700, 1650, 1240, 1150, 1080, 1040 and 850 cm^{-1}, high uv end absorption (ϵ_{230} 7000, ϵ_{210} 18,000, MeOH), CD curve (MeOH) λ_{max} 240 nm, $[\theta]$ +6300. The low-resolution MS exhibited M^+ at *m/e* 362 [not seen in high-resolution MS which displayed the first peak ($M^+ - C_5H_8O_2$, 2.5%) at *m/e* 262.1176 (calcd for $C_{15}H_{18}O_4$, 262.1159) and the base peak at 83 (C_5H_8O)].

Anal. Calcd for $C_{20}H_{26}O_6$: C, 66.28; H, 7.23; O, 26.49. Found: C, 66.05; H, 7.52; O, 26.15.

The lower band (**1a**) was recrystallized from ethyl acetate: yield 0.29 g; mp 141°; $[\alpha]^{22}D$ −22° (*c* 1.1, $CHCl_3$); ir bands at 3400, 1760, 1700, 1640, 1140, 1040, 960, and 870 cm^{-1}; uv end absorption (ϵ_{230} 7000, ϵ_{210} 18,000); CD curve λ_{max} 257 nm, $[\theta]$ −4990 (MeOH). It did not react with $NaIO_4$ or with acetone–toluenesulfonic acid. The low-resolution MS exhibited M^+ at *m/e* 378; this was not seen in the high-resolution MS which displayed the first peak ($M^+ - C_5H_8O_2$) at *m/e* 278.1171 (1.3%) (calcd for $C_{15}H_{18}O_5$, 278.1153); other significant peaks were at 260 (1.6%, $M^+ - C_5H_8O_2 - H_2O$), 164 (13.5%, $C_{10}H_{12}O_2$), and 83 (100, C_5H_7O).

Anal. Calcd for $C_{20}H_{26}O_7$: C, 63.48; H, 6.93; O, 29.60. Found: C, 62.96; H, 6.64; O, 29.52.

Acetyltifruticin (1b) and Acetyldeoxytifruticin (4b). Acetylation of 15 mg of **1a** with acetic anhydride–pyridine gave **1b** as a gum, ir bands at 3400, 1760, 1740, 1710, 1650, 1235, 1150, 1040, 910, 740 cm^{-1}. The low-resolution mass spectrum exhibited diagnostic peaks at *m/e* 420 (M^+), 378 ($M^+ - C_2H_2O$), 360 (M^+ –

$C_2H_4O_2$), 320 ($M^+ - C_5H_8O_2$), 260 ($M^+ - C_2H_4O_2 - C_5H_8O_2$), 243 ($M - C_5H_8O_2 - C_2H_4O_2 - OH$), 83 ($C_5H_7O$, base peak).

Anal. Calcd for $C_{22}H_{28}O_8$: mol wt, 420.1784. Found: mol wt, 420.1780 (MS).

Acetylation of 20 mg of **4a** gave 20 mg of **4b** as a gum, ir bands at 3400, 1760, 1740, 1700, 1650, 1235, 1140, 1030 and 920 cm^{-1}. The low-resolution MS gave significant ions at *m/e* 404 (M^+), 344 ($M^+ - C_2H_4O_2$), 304 ($M^+ - C_5H_8O_2$), 244 ($M^+ - C_2H_4O_2 - C_5H_8O_2$), 226 ($M^+ - C_2H_4O_2 - C_5H_8O_2 - H_2O$), 83 ($C_5H_7O$, base peak).

Anal. Calcd for $C_{22}H_{38}O_7$: C, 65.33; H, 6.98; O, 27.69. Found: C, 65.11; H, 6.66; O, 27.85.

Reaction of 5 with Methanol. TLC analysis of a solution of **5** in MeOH indicated partial conversion to a less polar substance. The product **7** was separated by preparative TLC on silica gel (benzene–ethyl acetate, 2:1) as a gum which had ir bands at 3400, 1760, 1700 (weaker than the band at 1760), 1650, 1230, 1120, and 1000 cm^{-1}; uv end absorption (ϵ_{210} 19,500); diagnostic peaks in low-resolution MS at *m/e* 392 (M^+), 374 ($M^+ - H_2O$), 342 ($M^+ - H_2O - CH_3OH$), 292 ($M^+ - C_5H_8O_2$), 260 ($M^+ - C_5H_8O_2 - CH_3OH$), 83 (C_5H_7O, base peak).

Anal. Calcd for $C_{21}H_{28}O_7$: mol wt, 392.1835. Found: mol wt, 392.1837 (MS).

MnO_2 Oxidation of 1a. A solution of 20 mg of **1a** in 5 ml of AR $CHCl_3$ was stirred with 100 mg of active MnO_2 until TLC indicated disappearance of starting material (10 hr), filtered, washed, dried, and evaporated at reduced pressure. The residue was purified by preparative TLC on silica gel (Bz–ethyl acetate, 1:1): yield 15 mg of **3**; mp 215–217°; ir bands at 3400, 1760, 1700 (double strength), 1650, 1240, 1150, and 1040 cm^{-1}; uv (MeOH) λ_{max} 235 nm (ϵ 9000), strong end absorption (ϵ_{210} 21,000); diagnostic peaks in the low-resolution MS at *m/e* 376 (M^+), 358 ($M^+ - H_2O$), 276 ($M^+ - C_5H_8O_2$), 259 ($M^+ - C_5H_8O_2 - OH$), 83 (C_5H_7O, base peak).

Anal. Calcd for $C_{20}H_{24}O_7$: mol wt, 376.1522. Found: mol wt, 376.1519 (MS).

Conversion of 1a to 2a. A solution of 80 mg of **1a** in 5 ml of anhydrous MeOH was allowed to stand for 4 hr with 100 mg of MeONa (nitrogen atmosphere), diluted with water, and extracted with ethyl acetate. The washed and dried residue was evaporated and the residue purified by preparative TLC on silica gel ($CHCl_3$–MeOH, 20:1) to provide 20 mg of gummy **2a**, ir bands at 3400, 1760, 1050, and 980 cm^{-1}. Acetylation of 10 mg of **2a** gave **2b**, which did not crystallize: ir bands at 1760, 1735, 1240, 1030, and 980 cm^{-1}; diagnostic peaks in the low-resolution MS at *m/e* 412 (M^+), 370 ($M^+ - C_2H_2O$), 352 ($M^+ - C_2H_4O_2$), 310 ($M^+ - C_2H_4O_2 - C_2H_2O$), 292 ($M^+ - 2C_2H_4O_2$), 43 ($C_2H_3O$, base peak).

Anal. Calcd for $C_{20}H_{28}O_9$: mol wt, 412.1733. Found: mol wt, 412.1729 (MS).

Epoxidation of 4a and 4b. A solution of 4 mg of **4a** in 2 ml of $CHCl_3$ was allowed to stand, with stirring, with 25 mg of *m*-chloroperbenzoic acid for 1 hr, diluted with water, and extracted with $CHCl_3$. The washed and dried extract was evaporated and the residue purified by preparative TLC (Bz–ethyl acetate, 1:1). The product was identical with **1a** in every respect. Similarly, **4b** afforded **1b**.

When the reaction time was extended, a mixture of products resulting from epoxidation of ring and ester side chain double bonds was obtained.

MnO_2 Oxidation of 4a. A solution of 10 mg of **4a** in 3 ml of AR $CHCl_3$ was stirred with 50 mg of active MnO_2, the reaction being monitored by TLC. When all of **4a** had disappeared (3 hr), the mixture was filtered, washed, dried, and evaporated at reduced pressure. The residue was purified by TLC on silica gel (Bz–ethyl acetate, 2:1). This gave **5** as a gum: wt 7 mg; ir bands at 3400, 1760, 1700, 1650 (very strong), 1250, 1130, 1040, 950 cm^{-1}; uv (MeOH) λ_{max} 250 nm (ϵ 8500); the low-resolution MS gave significant peaks at *m/e* 350 (M^+), 260 ($M^+ - C_5H_8O_2$), 243 ($M^+ - C_5H_8O_2 - OH$), 83 (C_5H_7O, base peak).

Anal. Calcd for $C_{20}H_{24}O_6$: mol wt, 360.1573. Found: mol wt, 360.1570 (MS).

Extraction of *Tithonia rotundifolia*. Above-ground parts (wt 13.5 kg) of *T. rotundifolia* (Mill.) Blake, collected by E. L. Tyson (Tyson no. 6446) on Nov 27, 1971 midway between Chorrera and Capira, Panama, along the Interamerican Highway, was extracted with $CHCl_3$ and worked up as usual.[15] The crude gum, wt 20 g, was chromatographed over 700 g of silicic acid, 500-ml fractions being collected in the following order: 1–10 (Bz), 11–20 (Bz–$CHCl_3$, 10:1), 21–30 (Bz–$CHCl_3$, 1:1), 31–40 (Bz–$CHCl_3$, 1:10), 41–50 ($CHCl_3$), and 51–60 ($CHCl_3$–MeOH, 20:1). Fractions 29–45, which showed the same two spots on TLC, were combined and the two substances were separated by preparative TLC (five 20 × 40 cm plates, silica gel, solvent Bz–ethyl acetate, 2:1). The less polar compound (probably **9b**) was recrystallized from ethyl acetate: yield 0.5 g; mp 125°; $[\alpha]^{22}D$ −55° (*c* 1.2, $CHCl_3$); ir bands at 1760, 1730, 1650, 1250, 1150, and 1040 cm^{-1}. The low-resolution MS exhibited significant peaks at *m/e* 380 (M^+), 292 ($M^+ - C_4H_8O_2$), 264 ($M^+ - C_4H_8O_2 - C_2H_4$), 246 ($M^+ - C_4H_8O_2 - C_2H_4 - H_2O$), 71 ($C_4H_8O$, base peak).

Anal. Calcd for $C_{21}H_{32}O_6$: C, 66.29; H, 8.48; O, 25.23. Found: C, 65.72; H, 8.47; O, 24.94.

The more polar compound (probably **9a**) was recrystallized from ethyl acetate: yield 3.1 g; mp 141°; $[\alpha]^{22}D$ −77° (*c* 2.0, $CHCl_3$); CD curve (MeOH) δ_{max} 263 nm, $[\theta]$ −1560; ir bands at 3400, 1760, 1735, 1650, 1230, 1150, 1030, and 960 cm^{-1}; significant peaks in the low-resolution MS at *m/e* 352 (M^+), 334 ($M^+ - H_2O$), 264 ($M^+ - C_4H_8O_2$), 246 ($M^+ - C_4H_8O_2 - H_2O$), 71 (C_4H_7O, base peak).

Anal. Calcd for $C_{19}H_{28}O_6$: C, 64.75; H, 8.01; O, 27.24. Found: C, 64.30; H, 7.72; O, 26.80.

Registry No.—**1a**, 56377-69-6; **1b**, 56377-59-4; **2a**, 56377-60-7; **2b**, 56377-61-8; **3**, 56377-62-9; **4a**, 56377-63-0; **4b**, 56377-64-1; **5**, 56377-65-2; **7b**, 56377-66-3; **9a**, 56377-67-4; **9b**, 56377-68-5.

References and Notes

(1) This work was supported in part by Grant CA-13121 from the U.S. Public Health Service through the National Cancer Institute.
(2) H. Yoshioka, W. Renold, and T. J. Mabry, *Chem. Commun.*, 148 (1970).
(3) For a similar situation in the oxidation of erioflorin,[4] which has a cis 4,5 double bond,[5] to dehydroerioflorin, see ref 4.
(4) S. J. Torrance, T. A. Geissman, and M. R. Chedekel, *Phytochemistry*, **8**, 2381 (1969).
(5) W. Herz and S. V. Bhat, *J. Org. Chem.*, **37**, 906 (1972).
(6) S. Gnecco, J. P. Poyser, M. Silva, and P. G. Sammes, *Phytochemistry*, **12**, 2469 (1973).
(7) S. Iriuchijima, S. Kuyama, N. Takahashi, and S. Tamura, *Agric. Biol. Chem.*, **30**, 1152 (1966).
(8) Orizabin[9] presumably falls in this category as well because of its resemblance to woodhousin,[5] although it was originally formulated as a C-4, C-5 *trans*-germacranolide.
(9) A. Ortega, C. Guerrero, A. Romo de Vivar, J. Romo, and A. Palafox, *Rev. Latinoam. Quim.*, **2**, 38 (1971).
(10) The impression conveyed by cursory inspection of formulas **7a** and **7b** that they differ in configuration at C-10 is erroneous, because C-10 is rotated through the plane of the page in going from **7a** to **7b** and vice versa.
(11) W. Herz and R. P. Sharma, *Phytochemistry*, in press.
(12) W. Stöcklin, T. G. Waddell, and T. A. Geissman, *Tetrahedron*, **26**, 2397 (1970).
(13) Z. Samek, *Tetrahedron Lett.*, 671 (1970).
(14) W. Herz and I. Wahlberg, *J. Org. Chem.*, **38**, 2485 (1973).
(15) The rules of Stöcklin et al.[12] and Samek[13] are applicable to germacranolides which do not contain a double bond in the ten-membered ring.
(16) W. Herz, A. Srinivasan, and P. S. Kalyanaraman, *Phytochemistry*, **14**, 233 (1975).
(17) W. Herz and G. Högenauer, *J. Org. Chem.*, **27**, 905 (1962).