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SESQUITERPENE LACTONES FROM *ARTEMISIA*: ARBUSCULIN-C, ROTHIN-A AND ROTHIN-B

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Abstract—Three new santanolides, arbusculin-C (III), rothin-A (VIII), and rothin-B (XII), were isolated from species of the section Tridentatae Rydb. of the genus *Artemisia*. Their structural details were revealed by means of NMR spectroscopy, and diagnostically useful examples of intramolecular proximity deshielding by oxygen were observed. Biosyntheses of these and other eudesmanolides from *A. arbuscula* Nutt. ssp. *arbuscula* and *A. rothrockii* Gray were rationalized in two analogous pathways from assumed germacranolide precursors.

INTRODUCTION

THE NORTH American species, commonly known as 'western sagebrush', of the section Seriphidium of the genus *Artemisia* (Compositae) have been classed as the section Tridentatae Rydb.[1] Several members of the Tridentatae have been investigated in this laboratory,[2,3] of which two chemically similar species *A. arbuscula* Nutt. ssp. *arbuscula* and *A. rothrockii* Gray have yielded thus far sesquiterpenes only of the eudesmane skeletal type. The structures of arbusculin-A (V), -B (I), and -E and the isolation of arbusculin-C (III) and -D from *A. arbuscula* ssp. *arbuscula* were previously reported.[3] Arbusculin-A and -C and rothin-A (VIII) and -B (XII) have now been isolated from *A. rothrockii* and the structures of the latter three compounds determined. Arbusculin-B was also isolated from a plant identified as *A. cana* ssp. *viscidula* (Osterhout) Beetle.

RESULTS AND DISCUSSION

Arbusculin-C (III) (Chart I), $C_{15}H_{20}O_3$, m.p. 150–151°, had i.r. absorption at 3555 (OH), 1765 (γ-lactone), 1672 (11-methylene), and 1745 cm^{-1} (4-methylene); and prominent mass spectral fragment ions of *m/e* 250 (M^+), and M-15, and M-18. The appearance of its NMR spectrum (Table 1) was that of a santanolide. The 11 Hz (*trans*) coupling between the doublet (δ 4·27) and the multiplet (δ 3·37) corresponding, respectively, to the 6β-H and the 7α-H, and the negative Cotton effect at 253 nm[4] were taken as evidence for the 6,7-*trans*-fused lactone. The structure was confirmed by the transformation of arbusculin-B (I) into arbusculin-C (III).

* Contribution number 2603, from the Department of Chemistry, UCLA, California, U.S.A.

[1] A. A. BEETLE, *Rhodora* **61**, 82 (1959).

[2] (a) T. A. GEISSMAN, T. STEWART and M. A. IRWIN, *Phytochem.* **6**, 901 (1967).
(b) M. A. IRWIN and T. A. GEISSMAN, *Phytochem.* **8**, 305 (1969).
(c) T. A. GEISSMAN, T. S. GRIFFIN and M. A. IRWIN, *Phytochem.* **8**, 1297 (1969).
(d) M. A. IRWIN, K. H. LEE, R. F. SIMPSON and T. A. GEISSMAN, *Phytochem.* **8**, 2009 (1969).
(e) K. H. LEE, R. F. SIMPSON and T. A. GEISSMAN, *Phytochem.* **8**, 1515 (1969).

[3] M. A. IRWIN and T. A. GEISSMAN, *Phytochem.* **8**, 2411 (1969).

[4] T. G. WADDELL, W. STÖCKLIN and T. A. GEISSMAN, *Tetrahedron Letters* 1313 (1969). Circular dichroism measurements were made by courtesy of T. G. Waddell.

Arbusculin-B (I), whose structure has been established by correlation with 'santanolide c',[3] yielded arbusculin-B oxide (4,5-epoxydihydroarbusculin-B) (II) upon treatment with *m*-chloroperbenzoic acid. The oxide, formed by the attack of the peracid at the least hindered face of the molecule, was assumed to have the α-configuration. One of the products of the acid-catalyzed rearrangement of arbusculin-B oxide was arbusculin-C (III).

Rothin-A (VIII), $C_{15}H_{20}O_3$, m.p. 133–134°, had i.r. absorption at 3580 (OH), 1764 (γ-lactone), 1667 cm^{-1} (11-methylene); prominent mass spectral fragment ions of *m/e* 248 (M^+), M-15, M-18, and M-15-18; and a negative Cotton effect at 258 nm. Its NMR

TABLE 1. NMR SPECTRA OF *Artemisia* SESQUITERPENE LACTONES (60 MHz)*

Compound	Substituent, except as noted				
	4		5α-OH	6β-H	7α-H
I	CH_3:	1·86 (br)	—	4·52 ($11·3/W_{1/2} = 5·5$)	2·53 (m/w = 32)
I*	CH_3:	1·89 (1·4)	—	4·55 ($11·2/W_{1/2} = 5$)	2·45 (m/w = 31)
II	CH_3:	1·56	—	4·35 (11·1)	3·00 (m/w = 32)
III	CH_2:	4·99 (1 2-m)	2·03	4·27 (11·1)	3·37 (m/w = 32)
III*	CH_2:	4·95 (1 5-t) 5·05 (1·5-t)	6·95 ($W_{1/2} = 2$)	4·37 (11·1)	3·64 (m/w = 32)
IV	CH_2:	4 72 (1·5-t) 4·94 (1·5-t)	2·27 ($W_{1/2} = 4$)	4·21 (10·1) α-OH: 2·27 ($W_{1/2} = 4$)	
VI	β-CH_3: α-$OSi(CH_3)_3$:	1·33 0 133	H: 1·80 (11·3)	3 95 (11·1/10·3)	2·53 (10·5-t/3-t/÷)
VIII	CH_3·	1·85 (1)	—	4 54 ($11·3/W_{1/2} = 6$)	2·61 (11·6/9·9/3·0-t)
VIII*	CH_3:	1·92 (1)	—	4·71 ($11·6/W_{1/2} = 5$)	2·81 (11·7/9·9/3·1-t)
IX	CH_3:	1·88 (1)	—	4·62 ($11·7/W_{1/2} = 5\ 5$)	2 84 (11·7/10 8/3·1-t)
IX*	CH_3:	1·90 (1)	—	4·78 ($11·5/W_{1/2} = 6$)	2·89 (11·6/10·7/3 1-t)
X	β-CH_3:	0·98 (7·0)		3·94 (11·9/10·4)	
XI	β-CH_3:	1·00 (7·3)		4 01 (11·7/10·4)	
XII*	CH_2.	4·99 (1·5-t) 5·11 (1·5-t)	6·5 (br)	4·54 (10·9)	3·99 (10·9/10·1/3·0-t)
XIII	CH_2:	5 05 (m)	2·09 ?	4·36 (11·3)	3·64 (11·1-t/3 0-t)
XIII*	CH_2:	5·00 (1·5-t) 5·12 (1·5-t)	7·43 ($W_{1/2} = 3$)	4 63 (11·1)	4 04 (11·0-t/3·1-t)

TABLE 1.—*cont.*

Compound	Substituent, except as noted			
	8	10β-CH_3	11-CH_2	3 and 9
I		1·11	5·52 (3·0) 6·09 (3·2)	
I*		1·01	5·38 (3·0/0·6) 6·15 (3·2/0·6)	
II		1·16	5·46 (3·0) 6·13 (3·0)	
III		0·96	5·39 (3·2) 6·07 (3·3)	3α-H: 2·64 (m/w = 41)
III*		0·94	5·33 (3·2/0·6) 6·10 (3·3/0·6)	3α-H: 2·87 (m/w = 39)
IV		0·95	5·72 (0·9-t) 6·25 (1·0) CO_2CH_3: 3·76	
VI		0·96	5·35 (3·1/0·5) 6·03 (3·3/0·5)	
VIII	β-H: 4·07 (10·5-t/4·7)† α-OH: 3·17 ($W_{1/2}$ = 7)	1·12	6·09 (3·1/0·9) 6·18 (3·2/0·9)	
VIII*	β-H: 4·29 (10·3-t/4·5) α-OH: 6·33 (br)	1·09	6·33− (3·0/1·4?) 6·33+ (3·0/1·4?)	9β-H: 2·06 (12·9/4·6)
IX	β-H: 5·22 (10·8-t/4·8) α-$OCOCH_3$: 2·08	1·18	5·62 (3·0/0·5) 6·20 (3·2/0·5)	9β-H: 2·05 (12·7/4·7)
IX*	β-H: 5·39 (10·6-t/4·6) α-$COCH_3$: 2·10	1·10	5·68 (3·0/0·5) 6·23 (3·2/0·5)	9β-H: 2·06 (12·3/4·5)
X	β-H: 3·95 (m/w = 32) α-OH: 1·84	1·04	α-CH_3: 1·36 (6·9)	
XI	β-H: 5·12 (10·6-t/4·7) α-$OCOCH_3$: 2·04	1·10	α-CH_3: 1·23 (6·8)	
XII*	β-H: 4·45 (10·0-t/4·5) α-OH: 6·5 (br)	1·02	6·29 (3·0) 6·29 (3·0)	9β-H: 1·82 (12·3/4·5) 3α-H: 2·89 (m/w = 39)
XIII	β-H: 5·29 (10·7/7·8-t)‡ α-$OCOCH_3$: 2·09	1·03	5·54 (3·0) 6·14 (3·2)	9-H_2: 1·78 (7·7)‡ 3α-H: *ca.* 2·64 (m/w = 38)
XIII*	β-H: 5·53 (10·7/9·6/6·0) α-$OCOCH_3$: 2·12	1·03	5·63 (3·0) 6·20 (3·2)	9β-H: 1·81 (12·4/5·8) 3α-H: 2·87 (m/w = 40)

* Chemical shifts are reported in δ units (tetramethylsilane reference) and coupling constants (J), which were due to coupling with one other proton (doublet), except as noted (t = triplet, m = multiplet, br = broad, w = width), in Hz. Spectra had good resolution, were analyzed as first-order, and were measured in chloroform-d and in pyridine-d_5(*).

† Fine structure appeared after addition of D_2O.

‡ 7·8 ~ 7·7 = 0·5 ($J_{8,9\alpha} + J_{8,9\beta}$); non-first-order.

spectrum[5] differed from that of arbusculin-B (I) in the features associated with the 8α-OH. The most conspicuous feature and principal evidence for the 8α-OH group was the existence of unusually large geminal coupling between the protons of $=CH_2$, (C-13), and a paramagnetic shift of the higherfield 13-H (*trans* to the lactone), relative to the spectrum of arbusculin-B,[6] further support of its stereochemistry, the 8β-H in rothin-A (VIII) (δ 4·07), and its acetate (IX) (δ 5·52) was coupled by 11 Hz (*trans*) with the 7α-H and the 9α-H and by 5 Hz (*cis*) with the 9β-H. The 7α-H appeared as a simpler multiplet than that of arbusculin-B due to the lack of coupling with an 8α-H.

Rothin-A (VIII) yielded a tetrahydro-derivative (X), m.p. 118–119·5°, $[\alpha]_D$ + 21·6°, whose structure was based by analogy on that of tetrahydroarbusculin-B ('santanolide' c),[3] and whose gross features are supported by spectral data.[7] The increasing paramagnetic shift of 11α-CH_3 in the series, tetrahydroarbusculin-B (δ 1·19)–tetrahydrorothin-A acetate (XI) (δ 1·23)–tetrahydrorothin-A (X) (δ 1·36), was attributed to the influence of the proximate 8α-substituent, as it correlates with the increasing Van der Waal's radius (electron density) of the 8α-atom.[8] This effect, also observed in the santonin–artemisin acetate–artemisin series,[5] is related to the influence of the 8α-substituent on the 13-H in 11-methylene compounds[6] and is analogous to chemical shift-structure correlations in steroids.[9]

Rothin-B (XII), $C_{15}H_{20}O_4$, m.p. 254–256°, had i.r. absorption at 3460 and 3360 (OH), 1757 (γ-lactone), 1664 (11-methylene), and 1647 cm^{-1} (4-methylene); prominent mass spectral fragment ions of *m/e* 264 (M^+), M-15, M-18, M-15-18, and M-18-18; and a negative Cotton effect at 253 nm. The 6β-H, 7α-H, and 8β-H appeared as a second order system centered near δ 4 2 (in py.-d_5), the analysis of which was aided by the 100 MHz spectrum. The magnitudes of the couplings between the 6β-H (δ 4·54) and the 7α-H (δ 3·99), between the 7α-H and the 8β-H (δ 4·45, δ 5·53 in XIII) were 11 and 10 Hz, respectively, and corresponded to an all-*trans* configuration among the 6, 7, and 8-substituents. The appearance of the 8β-H signals of rothin-B were similar to those of rothin-A.

A comparison of the chemical shifts of the 7α-H of III, XII and XIII with those of I, VIII, and IX, respectively (Table 2), furnished evidence for the existence of the 5α-OH in

[5] The detailed structural identification of rothin-A and -B relies chiefly on the internal consistency of interpretation of the N.M.R. spectra in this and the preceeding paper.[3] Arguments similar to those in this paper were used in rationalizing the N.M.R. spectra of santonin, artemisin and their derivatives; J. T. PINHEY and S. STERNHELL, *Australian J. Chem.* **18,** 543 (1965).

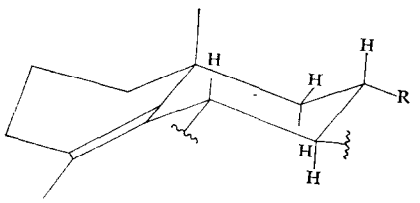
[6] A large and structurally diverse group of sesquiterpene 11-methylene lactones has been examined with respect to this phenomenon. It has been found in 6-closed lactones to be associated with 8α-oxygenation and increase in magnitude with the electron density at the oxygen atom (viz. 8α-$OCOCH_3$ < 8α-OH). The preliminary results of this study are to be published by H. YOSHIOKA, T. J. MABRY, Z. SAMEK and the present authors.

[7] A compound, m.p. 232°, $[\alpha]_D$ + 37·5°, reported to have the same structure (subject to the subsequent revision of 11β-CH_3 to 11α-CH_3 in compounds correlated with α-santonin) and prepared from artemisin by Clemmensen reduction and from arctiopicrin and balchanolide probably has, in view of its mode of formation, the thermodynamically more stable equatorial 4α-CH_3. (a) M. SUCHÝ, V. HEROUT and F. ŠORM, *Coll. Czech. Chem. Commun.* **24,** 1542 (1959) (b) V. HEROUT, M. SUCHÝ and F. ŠORM, *Coll. Czech. Chem. Commun.* **26,** 2612 (1961).

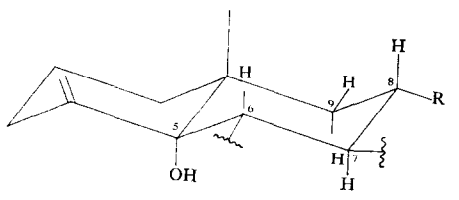
[8] Deshielding due to intramolecular steric compression (proximity) between groups which are separated by no more than the sum of their Van der Waal's radii has been reported in many molecules. See L. M. JACKMAN and S. STERNHELL, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, p. 71, Pergamon Press, Oxford (1969).

[9] Paramagnetic shifts (0·1–0·25 ppm) of the steroidal 18 and 19-CH_3 were correlated with 1,3-diaxially situated, 2, 4, 6, 8 or 11β-oxygenation. N. S. BHACCA and D. H. WILLIAMS, *Applications of NMR Spectroscopy in Organic Chemistry*, p. 29, Holden-Day, San Francisco (1964). The smaller shifts encountered in (X) and (XI) were explained by the divergence, required by the conformation of the five-membered ring, of the 11α-CH_3 and 8α-oxygen from a 1,3-parallel relationship.

TABLE 2. DESHIELDING OF 7α-H BY 5α-OH



type A



type B

R		A $\delta_{7\alpha\text{-H}}$ (c)	A $\delta_{7\alpha\text{-H}}$ (p)		B $\delta_{7\alpha\text{-H}}$ (c)	B $\delta_{7\alpha\text{-H}}$ (p)	shift $[\delta_A - \delta_B]_{7\alpha\text{-H}}$ (c)	shift $[\delta_A - \delta_B]_{7\alpha\text{-H}}$ (p)
H,	I:	2·53	2·45	III:	3·37	3·64	−0·84	−1·19
OH,	VIII:	2·61	2·81	XII:	—	3·99	—	−1·18
$OCOCH_3$,	IX:	2·84	2·89	XIII:	3·64	4·04	−0·80	−1·15

c = $CDCl_3$.
p = Pyridine-d_5.

the former group of compounds (type B; see Table 2). The large paramagnetic shifts of the 7α-H of type B-compounds was regarded as another example in these compounds of proxi mity deshielding,[8] in this case by the 5α-OH. Correlations in the steroid field supplied analogy for this system.[10] Further, of the two protons at C-9, of which only the 9β-H could be clearly discerned and its relative position determined by the second-order effect on peak heights, that at lower field is α (axial) (the reverse of the usual relationship is unperturbed cyclohexane annular methylene groups) in XII and XIII and β (equatorial) in VIII and IX.

The previously proposed biosynthesis of arbusculin-A (V) and -B (I)[3] may be expanded to rationalize the origins of arbusculin-C (III), rothin-A (VIII), and rothin-B (XII) (Chart II). The intermediacy of a cyclodecadiene in the biosynthesis of eudesmane-type sesquiterpenes has been put forward.[11] Costunolide (XIV) and hydroxycostunolide (XV) have been found in another *Artemisia* species,[7b,12] and the lack of evidence for the presence of unlactonized analogs of these compounds is consistent with the possibility that the lactone is carried through the pathway from an early stage. The steps of this pathway find parallels in *in vitro* reactions, i.e. the transannular cyclization of costunolide[11,13] and the synthesis of arbusculin-C from arbusculin-B. It cannot be inferred from the structures of the isolated compounds at what point in the pathway oxygen is introduced at C-8. The biochemical

[10] The magnitude of deshielding of a proton 1,3-diaxially juxtaposed with a hydroxyl group was found to be about half of that observed here. N. S. BHACCA and D. H. WILLIAMS, *Applications of NMR Spectroscopy in Organic Chemistry* p. 183, Holden-Day, San Francisco (1964). The larger magnitude seemed explicable, however, by a convergence, required by the conformation of the five-membered ring, the 7α-H and 5α-OH from a 1,3-diaxial relationship in type B-compounds.

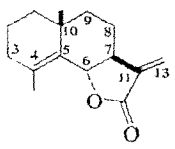
[11] J. B. HENDRICKSON, *Tetrahedron* **7**, 82 (1959); W. PARKER, J. S. ROBERTS and R. RAMAGE, *Quart. Rev.* **21**, 331 (1967).

[12] M. SUCHÝ, V. HEROUT and F. ŠORM, *Coll. Czech. Chem. Commun.* **28**, 1618 (1963).

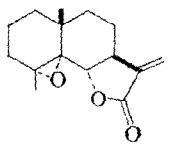
[13] T. C. JAIN and J. E. MCCLOSKEY, *Tetrahedron Letters* 2917 (1969).

interrelationships of many sesquiterpene lactones, several from this laboratory, as yet unpublished, can be consistently accounted for by reactions promoted by biological forms of electrophilic oxygen (represented here for convenience by hypothetical OH^+ but probably involving intermediate protonated epoxides) and electrophilic hydrogen (H^+) in simple cationic mechanisms. These types of reaction appear to be two of the predominant ones responsible for the diversity of sesquiterpene lactones.[11]

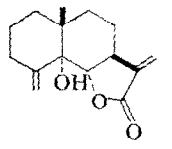
The chemical constitution of *A. arbuscula* ssp. *arbuscula* and *A. rothrockii* indicates that they have a common pathway for the biosynthesis of sesquiterpene lactones, and consequently supports a close phylogenetic relationship within the Tridentatae. Their difference, the ability to introduce oxygen at C-8, is minor compared to differences in composition found in other members investigated of the Tridentatae.[2,3] An examination with regard to season and geography to determine the variability of the chemical constitution of each species, however, would be necessary to thoroughly establish their relationship.



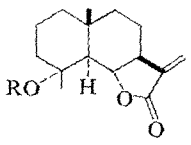
(I) arbusculin B



(II)

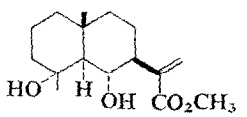


(III) arbusculin C

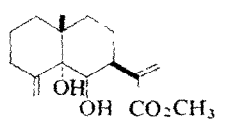


(V) R=H arbusculin A

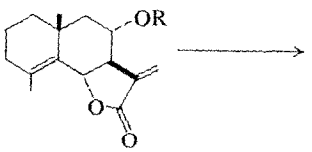
(VI) R=$Si(CH_3)_3$



(VII)

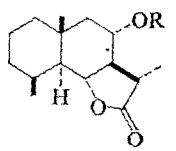


(IV)



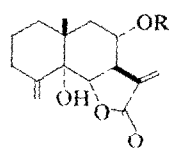
(VIII) R=H rothin A

(IX) R=$COCH_3$



(X) R=H

(XI) R=$COCH_3$



(XII) R=H rothin B

(XIII) R=$COCH_3$

CHART I. *Artemisia* SESQUITERPENE LACTONES.

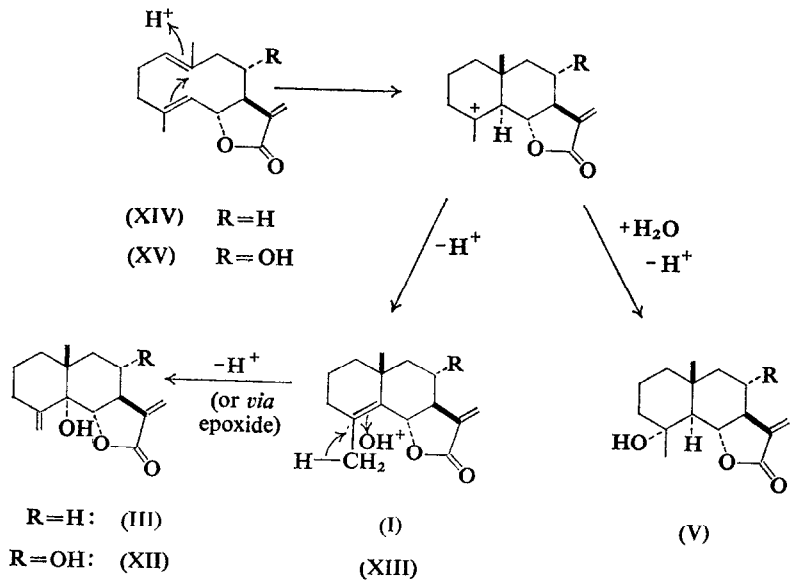


CHART II. SUGGESTED BIOGENETIC RELATIONSHIPS OF SOME *Artemisia* SESQUITERPENE LACTONES.

EXPERIMENTAL

TLC was done with silica gel G-coated plates developed in acetone–$CHCl_3$ and stained with H_2SO_4 spray. Spots appeared on warming of the plate; their colors, which are useful for identification, are reported. Corrected m.ps were obtained in glass capillaries. I.r. spectra were measured in $CHCl_3$, unless otherwise noted; mass spectra with an AEI MS-9 at 70 eV by direct insertion; u.v. spectra in ethanol; circular dichroism in methanol and NMR spectra with Varian A-60D and HA-100 instruments. NMR data appear in the Table.

Comparison of Plant Collections

Six plant collections within the section Tridentatae, including two that were worked-up (designated by *), whose extracts had similar thin-layer chromatograms indicating the presence of arbusculin-A (-C?), -B, and -D, are listed below. The collection of *A. cana* ssp. *viscidula* differed from the others in containing a large amount of a compound tentatively identified by its distinctive behavior on TLC as either matricarin or deacetoxymatricarin. The former has been found in *A. cana* Pursh ssp. *cana*[2e] and the latter has been observed to be typical of some *A. tridentata* subspecies (Ref. 2a and unpublished observations). Another collection of *A. cana* ssp. *viscidula* (voucher no. ROA-70067-ACV, 4 kg, collected near Laramie, Wyoming, June 1967)

Voucher No.	Dry Weight (kg)	Identification/Location
AAB-966-AAA*	(1·3)	*A. arbuscula* Nutt. ssp. *arbuscula*. Grand Teton National Park
ROA-90767-AAAR	(0·18)	*A. arbuscula* Nutt. ssp *arbuscula*. Grand Teton National Park
GHW-966-AAT	(0·09)	*A. arbuscula* ssp. *thermopola* Beetle. Snake River, north of Grand Teton
AAB-966-AAT	(0·61)	*A. arbuscula* ssp. *thermopola* Beetle. Flag Ranch
AAB-966-ACV*	(1·6)	*A. cana* ssp. *viscidula* (Osterhout) Beetle. Laramie
GHW-966-ATRS	(0·10)	*A. tripartita* ssp. *rupicola* Beetle. ('shade form') Pole Mountain

and several well-studied collections of *A. tripartita* ssp. *rupicola*[2b] were not similar to these. The third and fourth collections in this list were mistakenly reported as *A. arbuscula* ssp. *arbuscula* in the previous paper.[3] The collections were made in Wyoming, September 1966 (except the second, September 1967), at the indicated locations.

Arbusculin-C (III)

The isolation of arbusculin-C (III), AAA-1, from *A. arbuscula* ssp. *arbuscula* (voucher no. AAB-966-AAA) has been described.[3] The analytical sample was recrystallized from ether–EtOAc (fine needles) and had m.p. 150–151°; $[\alpha]_D^{25}$ + 113 (c = 3·06, chlf.); circular dichroism $[\theta]_{253}$ −3620; i.r., spectrum 3555 (m), 1765 (s), 1672 (w), 1645 cm^{-1} (w); m.s. *m/e* 248 (M^+, base), 233, 230, 214, 203, 201, 124; TLC blue-gray. (Found: C, 72·60; H, 8·34. Calcd. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12.)

Isolation of Arbusculin-B (I) and ACV-1 from A. cana *ssp.* viscidula

Dry, milled plant identified as *A. cana.* ssp. *viscidula* (Osterhout) Beetle (1·6 kg, voucher no. AAB-966-ACV) was extracted with $CHCl_3$ (3 × 4 l.), and a slurry of the concentrated extract in methanol–water (1·5 l., 3:1) was extracted with hexane (2 × 1·5 l.). The hexane extract was concentrated and chromatographed over a column (8 cm × 43 cm) of silica gel eluted with hexane–$CHCl_3$ (1:1). Fractions 3 and 4 (0·5 l.), containing arbusculin-B (I), ACV-2, were combined, concentrated, treated with activated charcoal and crystallized from petroleum at dry ice temp. giving 589 mg (0·038% yield) of crystals, m.p. 85–87°; identical (mixed m.p., i.r.) with arbusculin-B (I), AAA-2, from *A. arbuscula* ssp. *arbuscula* (m.p. 86·5–87°).[3] Fractions eluted just prior to those containing arbusculin-B yielded 28 mg of a crystalline compound (needles), ACV-1, having m.p. 145–148; i.r. 1705 cm^{-1} (s); m s. *m/e* 426 (M^+); NMR (chlf.-d) all signals higher field than δ 2·5, complex methyl region.

Isolation of Rothin-A (VIII) and -B (XII), ARO-5, and Arbusculin-A (V) and -C (III) from A. rothrockii

Dry, milled *A. rothrockii* (0·78 kg, voucher no. ROA-90767-ARO collected at Blind Bull Creek, Bridger National Forest, Wyoming, September 1967) was extracted with $CHCl_3$ (3 × 2·5 l.). The concentrated extract was slurried in methanol–water (2 l., 3:1) and extracted with hexane (1 l.). The methanol–water phase was evaporated under vacuum to 0·3 l. and extracted with $CHCl_3$ (4 × 0·5 l.). The concentrated extract was chromatographed over a column (8 × 37 cm) of silica gel eluted with $CHCl_3$–benzene and gradually increasing proportions of acetone in $CHCl_3$–benzene in 30 fractions (0·5 l. ea.).

Fractions 6–10, combined and concentrated, yielded, with the aid of ether, 1·26 g (0·16% yield) of rothin-A (VIII), ARO-1. The analytical sample (fine rectangular crystals), recrystallized from ether–EtOAc, had m.p. 133–134°; $[\alpha]_D^{25}$ + 122° (c = 5·55, chlf.); c.d. $[\theta]_{258}$ − 2120; u.v. end absorption; i.r. 3580 (m), 3450 (m, br), 1764 (s), 1667 cm^{-1} (w); m.s. *m/e* 248 (M^+), 233 (base), 240, 215; TLC blue-green. (Found: C, 72 56; H, 8·15. Calc. for $C_{15}H_{20}O_3$: C, 72·55, H, 8·12.)

Fractions 14 and 15, combined and concentrated, yielded, with the aid of ether, 0·24 g (0·031% yield) of rothin-B (XII), ARO-2. The analytical sample (fine granular crystals), recrystallized from EtOAc at room temperature to avoid polymerization, had m.p. 254–256°; $[\alpha]_D^{25}$ + 242 (c = 1·45, methanol); c.d. $[\theta]_{253}$ − 3200; u.v. shoulder at $[\epsilon]_{208}$ 8800; i.r. (nujol) 3460 (s), 3360 (s), 1757 (s, doublet), 1664 (w), 1647 cm^{-1} (w); m s. *m/e* 264 (M^+), 249, 246, 231, 228, 217, 202, 175, 153; TLC yellow-brown. (Found: C, 67 97; H, 7·66. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H. 7·63.)

Fractions 11–13, after extraction with aq. Na_2CO_3 and chromatography, yielded about 0·4 g of a non-crystalline sesquiterpene, ARO-5.

Fractions 2–5 were combined, concentrated, and chromatographed over silica gel, eluted with benzene–$CHCl_3$. Fractions containing arbusculin-C (III), ARO-7, were combined, concentrated, and the products twice crystallized from EtOAc–ether–petroleum giving 47 mg of fine needles, m.p. 145–148°, identical (mixed m.p., i.r., NMR) with arbusculin-C described above. Fractions containing arbusculin-A (V), ARO-6, were combined, concentrated, and treated with pyridine–trimethylchlorosilane. The solvent was pumped off and the residue chromatographed over silica gel eluted with hexane–$CHCl_3$. The fractions containing trimethylsilylarbusculin-A (VI) were combined, concentrated, and crystallized from ether–petroleum at dry ice temperature. The crystalline material was dissolved in methanol containing a trace of HCl. The solution was diluted with absolute ethanol and evaporated under vacuum. The residue was crystallized from ether–petroleum giving 63 mg of the methyl ester derived from arbusculin-A (VII) (fine needles), m.p. 105–106°, identical (mixed m.p , i.r.) with the compound prepared from authentic arbusculin-A (m.p. 106–107°)

Arbusculin-B Oxide (II)

Arbusculin-B (273 mg) in $CHCl_3$ (10 ml) was treated with a solution of *m*-chloroperbenzoic acid (499 mg) in $CHCl_3$ (10 ml) at 0°. The solution was mixed with a dilute aqueous solution (50 ml) of K_2CO_3 and Na_2SO_3 and the mixture extracted with $CHCl_3$ (2 × 50 ml). The concentrated extract, containing mainly one product (TLC), was chromatographed over silica gel, eluted with benzene–$CHCl_3$. The arbusculin-B oxide-containing fractions yielded fine crystals (153 mg) from ether–petroleum having m.p. 117–120°; i.r. 1771 (s), 1668 cm^{-1} (w); m.s. *m/e* 248 (M^+), 230, 215, 205, 163 (base); TLC blue-gray. (Found: C, 72·33; H, 8 33. Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12.)

Preparation of Arbusculin-C (III) from Arbusculin-B Oxide (II)

Arbusculin-B oxide (135 mg) in acetic acid (3 ml) was treated with *p*-toluene-sulfonic acid (25 mg) for 3 hr at 0°. The thin-layer chromatogram of the solution exhibited mainly spots corresponding to arbusculin-C and arbusculin-B oxide. The solution was diluted with water (50 ml) and extracted with $CHCl_3$ (2 × 50 ml). The concentrated extract was chromatographed over a column of silica gel, eluted with benzene–$CHCl_3$, and the arbusculin-C-containing fractions were combined and concentrated. The NMR spectrum of the latter indicated the presence of arbusculin-C and other compounds. The sample, taken in ether, was seeded with arbusculin-C and the crystals obtained were recrystallized from ether–petroleum giving fine needles (10 mg), m.p. 148–149°, identical (mixed m.p., i.r.) with arbusculin-C described above.

Methanolized Arbusculin-C (IV)

A solution of arbusculin-C in methanolic HCl was allowed to stand. The solution was diluted with water and extracted with $CHCl_3$. The concentrated extract was chromatographed (silica gel, benzene–$CHCl_3$), and the compound-containing fractions were combined and concentrated to a crystalline residue which was characterized by its NMR spectrum.

Tetrahydrorothin-A (X)

A solution of rothin-A (100 mg) in EtOAc (3 ml) was added to a mixture of 10% Pd-C (95 mg) in EtOAc (20 ml) presaturated with H_2. H_2 was taken up over 66 min (23·5 ml, theory 19·3 ml) and the solution was filtered and concentrated to a residue which was crystallized twice from EtOAc–ether–petroleum giving loose blades (50 mg). It had m.p. 118–119·5°; $[\alpha]_D^{24}$ + 21·6° (c = 2·06, chlf.); i.r. 3580 (m), 2450 (m), 1778 cm^{-1} (s); m.s. *m/e* 252 (M^+), 250,[14] 237, 235, 232, 219, 208, 190, 164, 149, 135, 109; TLC brown (prolonged heating). (Found: C, 71·58; H, 9·49. Calc. for $C_{15}H_{24}O_3$: C, 71·39; H, 9·59.)

Rothin-A Acetate (IX)

Rothin-A was treated with pyridine–Ac_2O for 1 day, and the solvent was pumped off. The residue was chromatographed (silica gel, benzene–$CHCl_3$) and the compound-containing fractions were concentrated to a persistent oil which was characterized by its NMR spectrum (Table 1).

Rothin-B Acetate (XIII)

Rothin-B was treated with pyridine–Ac_2O for 1 day, and the solvent was pumped off. The residue was crystallized twice from ether–petroleum giving fine lustrous crystals having m.p. 174·5–175·5°; i.r. 3555 (m), 1772 (s), 1735 cm^{-1} (s); m.s. *m/e* 306 (M^+), 264, 246, 228, 217, 203, 153, 124.

Tetrahydrorothin-A Acetate (XI)

The mother liquor from the crystallization of tetrahydrorothin-A (X) was treated with pyridine–Ac_2O for 1 day. The solvent was pumped off and the residue chromatographed over silica gel eluted with $CHCl_3$–benzene. The compound-containing fractions were combined and evaporated to an oil which was characterized by its NMR spectrum (Table 1).

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[14] The peak at *m/e* 250 was believed to arise from decomposition of X in the mass spectrometer rather than to be the molecular ion of dihydrorothin-A formed from partial hydrogenation. In successive scans of the molecular ion region, the *m/e* 252 peak diminished greatly and a significant peak appeared at *m/e* 253 (probe temperature 105–120°). The NMR spectrum of X exhibited no peaks attributable to partial hydrogenation and was unchanged after exposure of the compound to hydrogen and catalyst for 1 day.