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SESQUITERPENE LACTONES FROM *AMBROSIA CONFERTIFLORA* (COMPOSITAE)

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Abstract—We describe the isolation and chemistry of four eudesmanolides, reynosin (III), santamarine (IV), α-epoxysantamarine (V), and isotelekin (VII) and the germacranolide parthenolide (VI) from *Ambrosia confertiflora* collected in Mexico. Most of the populations which afforded one or more of the eudesmanolides also contained the previously described germacranolides, tamaulipin-A (I) and -B (II). Reynosin is a new natural product and this is the first report of epoxysantamarine from a natural source. The determination of ther stereochemistry of the epoxide function as α in epoxysantamarine and the experimental details for the structure determination of tamaulipin-B are reported. Other populations of the species from southwest Texas and north-central Mexico yielded the pseudoguaianolide dilactones, psilostachyin (VIII), psilostachyin-B (IX) and -C (X). Confertin (XI) and peruvin (XII) were isolated from a population of *A. confertiflora* from Fresnillo, Zacatecas, Mexico.

INTRODUCTION

THE GENUS *Ambrosia* (Compositae) is remarkable for the number (more than thirty) and skeletal types of sesquiterpene lactones which it elaborates.[1] As part of our effort to use this chemical data for understanding evolutionary relationships among the species belonging to this genus, we are presently engaged in a broad investigation of the infraspecific variation of sesquiterpene lactones in a number of *Ambrosia* species.[1] In this connexion, we now report the detailed chemistry for several populations of *A. confertiflora* DC. which appear to be representatives of three of the four major chemical races in this species.

We previously reported[2] that two pseudoguaianolides, confertiflorin and desacetylconfertiflorin, characterized populations of *A. confertiflora* from south Texas and that the germacranolides tamaulipin-A (I)[3] and -B (II)[4] were the major constituents in populations of the species from near Cd. Victoria, Tamaulipas, Mexico. Also the pseudoguaianolides confertin and desacetylconfertiflorin have been isolated from a collection of the species obtained from central Mexico[5] and two pseudoguaianolide dilactones, psilostachyin and psilostachyin C, were detected in an Arizona population of the species.[6]

Most collections of *A. confertiflora* DC. from the Mexican state of Tamaulipas as well as from much of the state of Nuevo Leon were found to contain I and II mixed with two or three

* To whom inquiries regarding this paper should be addressed.

[1] T. J. MABRY, in *Phytochemical Phylogeny* (edited by J. B. HARBORNE), Academic Press, London (1970).
[2] N. H. FISCHER and T. J. MABRY, *Tetrahedron* **23**, 2529 (1967).
[3] N. H. FISCHER, T. J. MABRY and H. B. KAGAN, *Tetrahedron* **24**, 4091 (1968).
[4] N. H. FISCHER and T. J. MABRY, *Chem. Commun.* 1235 (1967).
[5] J. ROMO, A. ROMO DE VIVAR, A. VÉLEZ and E. URBINA, *Can. J. Chem.* **46**, 1535 (1968); [see J. ROMO, P. JOSEPH-NATHAN and G. SIADE, *Tetrahedron* **22**, 1499 (1966), for the optical rotation for confertin, i.e. anhydrocumanin].
[6] W. HERZ, G. ANDERSON, S. GIBAJA and D. RAULIS, *Phytochem.* **8**, 877 (1969).

eudesmanolides. One of the eudesmanolides (III) was found to be a new natural product and was named reynosin; the other two were santamarine (IV) and epoxysantamarine (V). We determined the stereochemistry of the epoxide function in V, which is reported here as a natural product for the first time although it previously had been described as a derivative of IV.[7] This is the first report of eudesmanolides from this species. Other populations of *A. confertiflora* which occur near Reynosa, Mexico, yielded the germacranolide parthenolide (VI) alone or mixed with various combinations of I–V.

Populations of the species from 100 miles northwest of Gomez Palacio, Chihuahua, Mexico, afforded the eudesmanolide isotelekin (VII) mixed with parthenolide. A large number of populations from southwest Texas and north-central Mexico were found to contain only the pseudoguaianolide dilactones, psilostachyin (VIII), psilostachyin-B (IX) and -C (X). The pseudoguaianolides confertin (XI) and peruvin (XII) were isolated from a population of *A. confertiflora* from Fresnillo, Zacatecas, Mexico.

We now recognize that the distribution of these sesquiterpene lactones (I–XII) and confertiflorin and desacetylconfertiflorin circumscribe three of the four major chemical races detected in this species. A description of the fourth chemical race along with the detailed chemical, morphological and, to some extent, cytological data for all of the more than 250 populations of *A. confertiflora* which we have investigated will be reported in a later publication.

RESULTS AND DISCUSSION

Reynosin (III)

The new eudesmanolide reynosin ($C_{15}H_{20}O_3$, m.p. 145–6°, $[\alpha]_D^{25} + 180°$) exhibited NMR, i.r. and u.v. spectral properties (see Table 1 and Experimental) which indicated that it might be structurally related to santamarine (IV), a eudesmanolide with which it co-occurs (see below). Significantly, a comparison of the NMR spectra of reynosin and santamarine indicated that the former contained an exocyclic double bond in contrast to the endocyclic double bond known to be present in IV. The presence of a secondary hydroxyl group in III was confirmed by the oxidative conversion of reynosin to dehydroreynosin (XIII).

Since the structure of santamarine is well established,[7] we attempted to confirm structure III for reynosin by converting santamarine and reynosin to the same tetrahydroderivative. Although some difficulties were encountered, the results did ultimately establish structure III for reynosin. Catalytic hydrogenation of santamarine with Pd-C as catalyst afforded tetrahydrosantamarine (XIV). However, when reynosin was hydrogenated under the same conditions it gave material (designated here as "x"; m.p. 165–169°), which was almost identical by NMR, m.p. and mixed m.p. with XIV but nevertheless differed from XIV in the fingerprint region of its i.r. spectrum. The hydrogenation of III with PtO_2 as catalyst gave a separable mixture of tetrahydroreynosin (XV), m.p. 170–173°, and dihydroisoreynosin XVI, m.p. 190–193°. Comparison of the i.r. spectra for XIV and XV with the one obtained for material "x" indicated that the latter might be a mixture of the former two substances in a 2:1 ratio. Preparation of an authentic mixture of XIV and XV in a 2:1 ratio confirmed this suggestion; the mixture of XIV and XV upon recrystallization gave crystals which were identical in all respects (NMR, i.r., m.p. and mixed m.p.) with "x". Both the "x" material and the 2:1 mixture of XIV and XV chromatographed as a single spot on silica gel G plates

[7] A. ROMO DE VIVAR and H. JIMENEZ, *Tetrahedron* **21**, 1741 (1965).

with several different solvent systems. The stereochemical assignments for the C-4 methyl groups in XIV and XV are based upon the fact that XIV was identical by i.r. and m.p. with dihydrobalchanin[8] which is known to correspond to structure XIV. Unfortunately, an authentic sample of dihydrobalchanin was not available for direct comparison.* Since

TABLE 1. NMR DATA FOR REYNOSIN, EPOXYSANTAMARINE, SANTAMARINE, PARTHENOLIDE AND DERIVATIVES*

Compounds	H_1	H_6	H_7	C_{10}-Me	C_{11}-Me	$C_{11}=CH_2$	Other
III	3·55 dd (4 and 10·5)	4·06 t (11)	2·52† c	0·83	—	5·45 d (3) 6·12 d (3·5)	4·90 5·03 brd‡ (W 1/2, 4)
IV	3·70 dd (6 and 9)	3·98 t (10·5)	—	0·88	—	5·43 d (3) 6·10 d (3)	5·40§ brd 1·85‖ brd (W 1/2, 3)
V	3·40 dd (3·5 and 10)	3·94 dd (10 and 11)	—	0·93	—	5·45 d (3) 6·13 d (3)	3·04 dd§ (3 and 1) 1·50‖
XIII	—	4·13 t (11)	—	1·11	—	5·47 d (3) 6·14 d (3)	5·15 5·25 brd‡ (W 1/2, 4)
XIV	3·33 c	4·04 brd, c	—	1·03	1·21 d (7)	—	1·00‖ d (7)
XV	3·32 c	3·88 brd, c	—	0·93	1·20 d (7)	—	1·03‖ (7)§
XVI	3·27 c	4·70 brd, d (10)	—	1·03	1·82 d (1·5)	—	1·08‖ d (6 ~ 7)
XVII	4·75 dd (6·5 and 10)	3·93 dd (10 and 11)	2·57† c	1·02	—	5·45 d (3) 6·14 d (3)	2·06** 1·50‖ 3·04 dd§ (3 and 1)

* Spectra were recorded in $CDCl_3$ with a Vairan A-60 spectrometer. Chemical shifts are given in ppm, δ-scale with TMS as an internal standard. Numbers in parentheses denote coupling constants in c/s. Signals are singlets unless otherwise stated: d, doublet; t, triplet; dd, doubledoublet; c, complex; brd, broad.

† These chemical shifts were determined by spin-decoupling.

‡ $C_4 = CH_2$.

§ H-3.

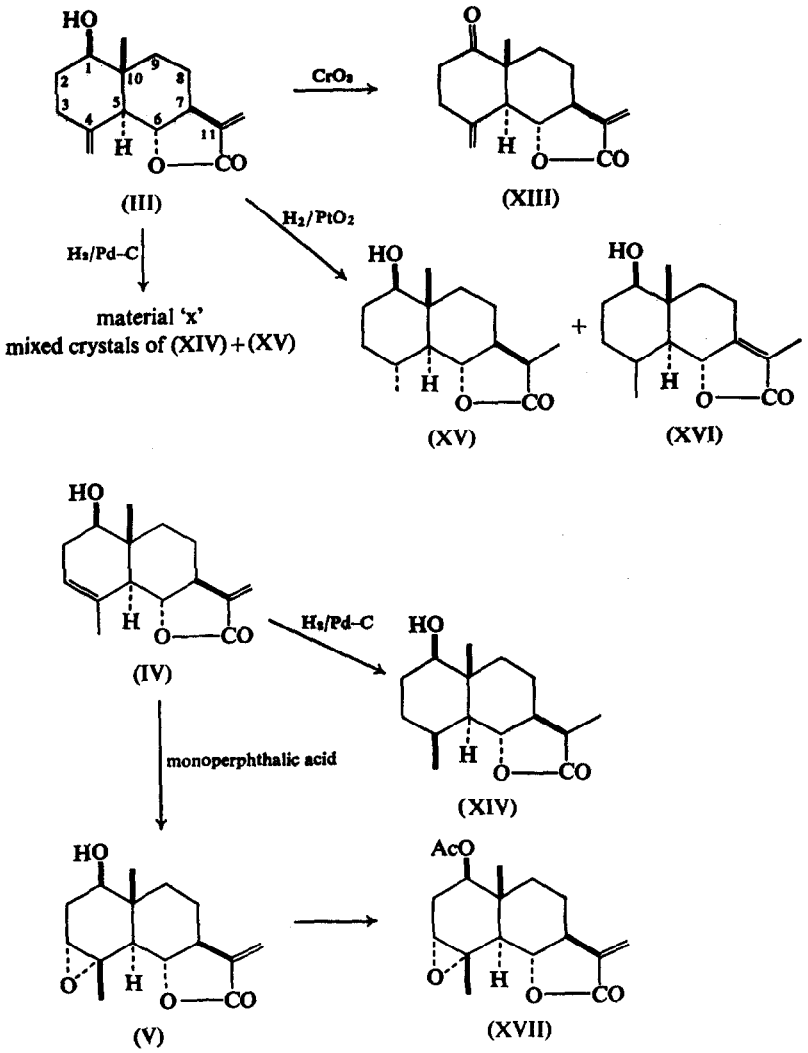
‖ C_4-Me.

** C-1 Acetate-Me.

compound XV differs with XIV only in stereochemistry at C-4, XV must be the C-4 α-methyl epimer.

* We thank Dr. V. Herout for the i.r. spectrum of dihydrobalchanin. Also Dr. Herout mentioned that the published[8] m.p. for dihydrobalchanin was incorrect; the correct m.p. (183°) agrees closely with the m.p. we observed for XIV.

[8] M. SŮCHY, *Coll. Czech. Chem. Commun.* **27**, 2925 (1962).



Santamarine (IV) and Epoxysantamarine (V)

One of the eudesmanolides [(IV); $C_{15}H_{20}O_3$; m.p. 134–135°] isolated along with reynosin from *Ambrosia confertiflora* was identical in all respects (NMR, i.r., m.p. and mixed m.p.) with an authentic sample* of santamarine, a sesquiterpene lactone previously isolated from *Chrysanthemum parthenium.*[7] Epoxidation of santamarine gave a substance identical with the third eudesmanolide [(V); $C_{15}H_{20}O_4$; m.p. 243–243·5°; $[\alpha]_D^{25}+87{\cdot}5°$] from *Ambrosia confertiflora;* this is the first report of epoxysantamarine as a natural product.

The previous report[7] of epoxysantamarine did not include a description of the stereochemistry of the epoxide function. We assign an α-orientation to the epoxide group on the basis of the following evidence: 1. molecular models for santamarine (IV) indicate that

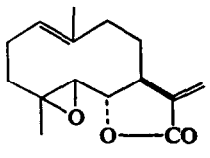
* We thank Dr. J. Romo for samples of santamarine, confertin and peruvin.

α-epoxidation is sterically favored; 2. the observed coupling constants between H-3 and the two C-2 protons were 1 and 3 c/s; these small coupling constants clearly required that H-3 be β-oriented (based upon an examination of molecular models for both α- and β-epoxysantamarine); 3. finally, Nuclear Overhauser effect studies carried out with epoxysantamarine acetate (XVII) showed a positive response between the C-4 methyl group and H-6 (17 per cent increase in the intensity of the signal for H-6 upon irradiation of the signal for the C-4 methyl group) indicating a *syn* relationship between them. A similar response was also observed between the C-10 methyl group and H-6.

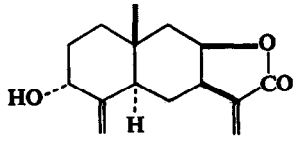
Parthenolide (VI) and Isotelekin (VII)

A single sesquiterpene lactone ($C_{15}H_{20}O_3$, m.p. 115–116°) was isolated from a population of *A. confertiflora* obtained near Reynosa, Mexico, in May 1967. The material corresponded to the known germacranolide parthenolide (VI), previously isolated from one species of Magnoliaceae: *Michelia champaca*[9] and two species of Compositae: *Chrysanthemum parthenium*[10] and *Ambrosia dumosa*.[11] Other populations from Tamaulipas, Mexico, contained parthenolide mixed with various combinations of I–V.

A population of *A. confertiflora* from 100 miles northwest of Gomez Palacio, Chihuahua, Mexico, afforded parthenolide mixed with a eudesmanolide (m.p. 143–144°) identical in all respects with an authentic sample of isotelekin* (VII). Isotelekin has previously been reported from the Compositae species *Telekia speciosa*.[12]



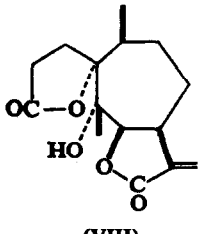
(VI)



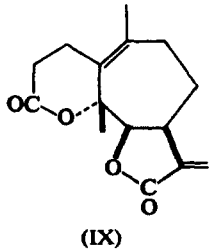
(VII)

Psilostachyin (VIII), Psilostachyin-B (IX) and Psilostachyin-C (X).

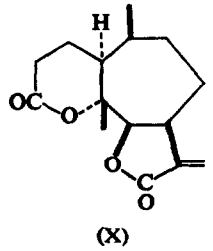
A number of populations of *A. confertiflora* from southwest Texas and in north-central Mexico were found by NMR to contain the pseudoguaianolide dilactones, psilostachyin (VIII), -B (IX) and -C (X), compounds previously isolated from a number of other *Ambrosia* species.[1] We isolated the three compounds from a collection of plants obtained near Piedras Negras, Coahuila, Mexico. This is the first report of psilostachyin-B from this species; however, VIII and X were previously detected in an Arizona population of *A. confertiflora*.[6]



(VIII)



(IX)



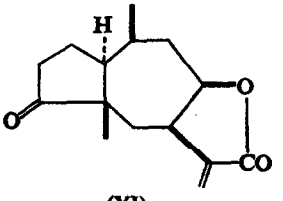
(X)

* We thank Dr. Miroslav Holub for data for isotelekin.

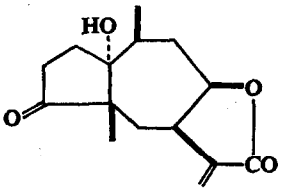
[9] T. R. Govindachari, B. S. Joshi and V. N. Kamat, *Tetrahedron* **21**, 1509 (1965).
[10] M. Soucek, V. Herout and F. Sorm, *Coll. Czech. Chem. Commun.* **26**, 803 (1961).
[11] T. A. Geissman and S. Matsueda, *Phytochem.* **7**, 1613 (1968).
[12] V. Benesova, V. Herout and F. Sorm, *Coll. Czech. Chem. Commun.* **26**, 1350 (1961).

Confertin (XI) and Peruvin (XII)

A population of *A. confertiflora* from Fresnillo, Zacatecas, Mexico, afforded the two known pseudoguaianolides, confertin (XI) and peruvin (XII). Confertin was previously found to co-occur with desacetylconfertiflorin in this species,[5] while peruvin has been isolated from *A. peruviana*[6,13] and *A. artemisiifolia*.[14]



(XI)



(XII)

Tamaulipin-B (II)

We presented most of the arguments regarding the structure assignment for tamaulipin-B in a preliminary communication.[4] Therefore, here we only briefly comment on the reaction sequences involved in the structure determination; all the data are presented in the Experimental and Table 2. Hydrogenation and acetylation of II gave dihydrotamaulipin-B acetate (XX) which on pyrolysis gave an equilibrium mixture* of XX and the Cope product XXI.

TABLE 2. NMR DATA FOR TAMAULIPIN-B AND DERIVATIVES*

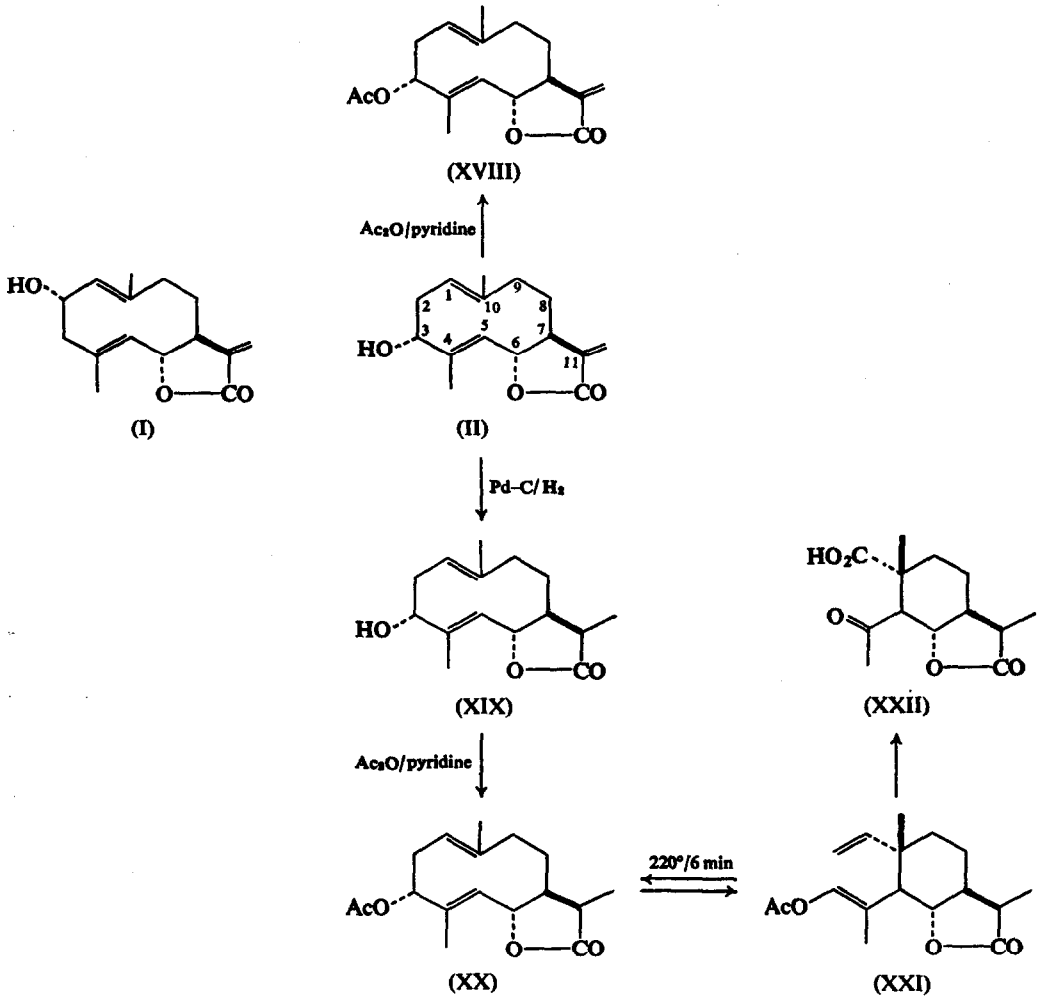
Compound	H_1 and H_5	H_3	H_6	C_4 and C_{10} methyl groups	C_{11}—CH_2 C_{11}—CH_3	Other signals
II	5·08brd 5·34brd $J_1 = 10·0$	4·50t (3·0)	4·65dd $J_1 = 8·0$ $J_2 = 10·0$	1·63d (1·2) 1·44d (1·0)	6·25d (3·5) 5·55d (3·2)	
XVIII	5·05c	5·36t (3·0)	4·61dd $J_1 = 8·5$ $J_2 = 10·0$	1·72d (1·5) 1·45d (1·0)	6·28d (3.5) 5·54d (3·1)	2·08 (Ac)
XIX	5·15c	4·47t (3·0)	4·63dd $J_1 = 8·0$ $J_2 = 10·0$	1·63d (1·5) 1·43d (1·0)	1·25d (6·5)	
XX		5·33t (3·0)	4·60dd $J_1 = 8·0$ $J_2 = 10·0$	1·71d (1·5) 1·45d (1·0)	1·26d (6·5)	2·08 (Ac)
XXI	H_1 5·84dd (10·0) (18·0) H_5 2·85d (12·0)	7·02t (1·5)	4·2brd	1·73d (1·5) 1·09 (C_{10})	1·25d (6·5)	2·08 (Ac) H_{2a} 4·90dd (10·0) (1·0) H_{2b} 4·91dd (18·0) (1·0)

* See footnote *, Table 1.

* A similar equilibrium mixture between a germacranolide and its Cope product was previously reported: K. TAKEDA, H. MINATO and M. ISHIKAWA, *J. Chem. Soc.* 4578 (1964).

[13] P. JOSEPH-NATHAN and J. ROMO, *Tetrahedron* **22**, 1723 (1966).
[14] T. H. PORTER and T. J. MABRY, *Phytochem.* **8**, 793 (1969).

The Cope product was subsequently converted to the known ketoacid[3] (XXII). The formation of XXII from XXI confirmed the assignment of structure II to tamaulipin-B (with the exception of the stereochemistry at C-3). The stereochemistry at C-3 in II is assigned an α-orientation on the basis of the recovery of dextrorotatory α-phenylbutyric acid in 77 per cent optical yield by the Horeau method.[2, 15]



EXPERIMENTAL*

Isolation of Tamaulipin-A (I), -B (II), Reynosin (III), Santamarine (IV), and Epoxysantamarine (V)

Ground whole plants of *Ambrosia confertiflora* DC. (voucher No. 260274),† collected 4 June 1967, 67 miles south of Reynosa on route 97 in the state of Tamaulipas, Mexico, were extracted with $CHCl_3$ and worked up

* M.p.s. are uncorrected. Analyses were determined by Dr. Alfred Bernhardt, Mikroanalytisches Laboratorium, Elbach über Engelskirchen, West Germany.

† All voucher specimens are deposited in The University of Texas Herbarium, Austin, U.S.A.

[15] (*a*) A. Horeau, *Tetrahedron Letters* 506 (1961) and 965 (1962); (*b*) T. J. Mabry, W. Renold, H. E. Miller and H. B. Kagan, *J. Org. Chem.* 31, 681 (1966).

in the usual way:[16] yield 36·3 g of crude syrup from 1·04 kg of plant material. The crude syrup was dissolved in ether–$CHCl_3$ (9:11) and chromatographed over a column of silica gel (1·1 kg, packed in the ether–$CHCl_3$ solvent). The column was successively eluted in 100-ml portions with five fractions of ether–$CHCl_3$ (9:11) and seventy fractions of ether–$CHCl_3$(1:1).

All fractions were monitored by TLC: Fractions 11–21 yielded 552 mg of crude tamaulipin-B (*II*). Fractions 32–42 yielded 719 mg of crude tamaulipin-A (*I*). Fractions 22–31 yielded 5·9 g of a viscous oil which was again chromatographed in a similar way over 130 g of silica gel with ether–$CHCl_3$ (9:11). Fractions 3–7 from the latter column yielded 950 mg of crude II and fraction 8–17 yielded 2·4 g of a mixture which yielded, after further chromatography, first 850 mg of I, next 600 mg of partially crystalline material, and finally 350 mg of II. Total yields of I and II were respectively 0·15% and 0·14% based on dry plant material.

The 600 mg of partially crystalline material yielded, after chromatography over $AgNO_3$–impregnated silica gel G plates, reynosin (III) and santamarine (IV). Compounds III and IV were also separable by fractional crystallization from benzene. The crude santamarine material, after recrystallization from isopropyl ether–acetone, gave pure santamarine (IV); m.p. 134–135°, identical in all respects with an authentic sample.*

The benzene mother liquor yielded, upon concentration, a second crop of crystals which were shown by NMR to be III and IV in a 1:2 ratio. When the filtrate from the second crop was taken to dryness, 200 mg of crude III were obtained; after recrystallization from isopropyl ether–acetone an analytically pure specimen of reynosin (III) was isolated; m.p. 145–146°, $[\alpha]_D^{25}$ + 180° (*c.* 0·12, EtOH); λ_{max} (EtOH): 210·5 nm (ϵ 9200); i.r. bands ($CHCl_3$): 3500, 1750, 905, 850, and 814 cm^{-1}. (Found: C, 72·74; H, 8·02; O, 19·37. $C_{15}H_{20}O_3$ required: C, 72·55; H, 8·12; O, 19·33.)

Fractions 55–75 from the first chromatographic run described above yielded 1 g of a viscous oil which afforded 300 mg of crude epoxysantamarine (V) (0·03% yield); m.p. 243·5–246° (from EtOAc), $[\alpha]_D^{25}$ + 87·5° (*c.* 0·096, EtOH), λ_{max} (EtOH): 212 nm (ϵ 8850); i.r. bands (nujol): 3380, 1748, 1670, 1135, 817 and 783 cm^{-1}. (Found: C, 68·05; H, 7·43; O, 24·32. $C_{15}H_{20}O_4$ required: C, 68·20; H, 7·58; O, 24·22.)

Isolation of Parthenolide (VI)

Ground leaves of *A. confertiflora* plant material collected on 4 May 1967, a half-mile north of the intersection of Highway 40 and 97, Reynosa, Tamaulipas, Mexico (voucher No. 255638),† were extracted and worked up as previously described: 20 g of plant material yielded 650 mg of crude syrup which afforded, after standing in ether, 240 mg (1·2% yield) of parthenolide, m.p. 115–116° (from $CHCl_3$–petroleum ether). The material was identical by NMR and m.p. with the published data[10] for parthenolide.

Isolation of Isotelekin (VII)

A 20 g (dry wt. for stems and leaves) collection of *A. confertiflora* (voucher No. 271768)† made on 5 June 1969, 100 miles north-west of Gomez Palacio in the state of Chihuahua on highway 49 towards Jimenez, Mexico, yielded 0·40 g of a crude syrup: NMR analysis indicated the presence of parthenolide and a eudesmanolide in a 1:1 ratio. Preparative thick-layer chromatography of the syrup (silica gel G; CH_2Cl_2:EtOAc; 1:1) yielded 60 mg of parthenolide. A more polar band gave 120 mg of a solid material which, after several recrystallizations from EtOAc–n-hexane, yielded 24 mg of needles, m.p. 144°; $[\alpha]_D^{25}$ + 93° (c. 5·19, $CHCl_3$). The material was identical in all respects with isotelekin.[12]

Isolation of Psilostachyin (VIII), Psilostachyin-B (IX) and Psilostachyin-C (X)

A collection of *A. confertiflora* made on 4 July·1968 about 30 miles south of Piedras Negra, Coahuila, Mexico (voucher No. 261652),† yielded by standard procedures the three dilactones in a 1:3:1 ratio: psilostachyin (VIII), m.p. 215°, psilostachyin-B (IX), m.p. 123°, and psilostachyin-C (X), m.p. 223–225°. The compounds were identical in all respects with authentic samples.

Isolation of Confertin (XI) and Peruvin (XII)

A collection of 63 g (dry wt. for stems and leaves) of *A. confertiflora* (voucher No. 271776)† made on 4 June 1969, 0·6 miles northwest of Fresnillo (State of Zacatecas) on highway 45, yielded 2·13 g of a crude, partially crystalline syrup: NMR and TLC analysis indicated the presence of peruvin as the major and confertin as the minor components. A yield of 550 mg of crystals, m.p. 149–158°, was obtained by extracting the oil away from the crystals with ether. Recrystallization from acetone–n-hexane yielded eventually pure peruvin, m.p. 154–160° (further recrystallizations did not raise the m.p.), $[\alpha]_D^{25}$ + 155° (*c.* 5·26, $CHCl_3$). The material was identical by i.r. and NMR with an authentic sample* of peruvin. Samples of peruvin have been reported[13,14] to melt higher; however, all workers have encountered difficulties in obtaining a consistent melting point for this substance.

When the mother liquor from the solution which yielded peruvin was cooled to −20° for several hours, confertin crystallized out. A final purification by preparative thick-layer chromatography (silica gel G;

[16] T. J. MABRY, H. E. MILLER, H. B. KAGAN and W. RENOLD, *Tetrahedron* **22**, 1139 (1965).

CH_2Cl_2:EtOAc, 1:1) and recrystallization from acetone–n-hexane yielded pure confertin, m.p. 145–146°, $[\alpha]_D^{25}$ + 179° (*c.* 2·68, $CHCl_3$). The material was identical with an authentic sample of confertin[5],* by m.p., mixed m.p., i.r. and NMR.

Oxyreynosin (XIII)

Three drops of CrO_3 reagent[17] were added at room temperature to 30 mg of III in 0·5 ml of acetone. After 0·5 min the mixture was diluted with 2 ml of water and extracted with two 3-ml portions of $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, aq. sat. $NaHCO_3$ and again with H_2O and then dried over anhyd. Na_2SO_4. Evaporation of the $CHCl_3$ left 25 mg of crude XIII, which was recrystallized from isopropyl ether; m.p. 141·5–144°; i.r. bands ($CHCl_3$): 1770, 1710, 1650, 910, 848, and 813 cm^{-1}. (Found: C, 73·01; H, 7·46; O, 19·39. $C_{15}H_{18}O_3$ required: C, 73·22; H, 7·33; O, 19·50.)

Dihydroisoreynosin (XVI) and Tetrahydroreynosin (XV)

A solution of 100 mg of III in 15 ml of glacial acetic acid was hydrogenated in the presence of 35 mg of PtO_2 as catalyst. (The catalyst had been prehydrogenated for 20 min.) Hydrogen uptake was 20 ml (*ca.* 2 mole equivalents with respect to compound III) after 25 min. After filtration of the catalyst the solution was taken to dryness *in vacuo*, and the residue was dissolved in 5 ml of $CHCl_3$. The $CHCl_3$ solution was washed with aq. sat. $NaHCO_3$ and dried over anhyd. Na_2SO_4. Evaporation of the $CHCl_3$ afforded a solid residue which furnished 40 mg of crystals upon trituration with 0·5 ml of ether. The crystals were preparatively chromatographed over silica gel G by double development with benzene–ethyl acetate (3:1); one band (R_f 0·31) yielded 15 mg of tetrahydroreynosin (XV), m.p. 170–173°; i.r. band ($CHCl_3$): 3580, 1750, 989, and 914 cm^{-1}. (Found: C, 71·17; H, 9·67. $C_{15}H_{24}O_3$ required: C, 71·50; H, 9·53.)

A second band (R_f 0·21) yielded 15 mg of dihydroisoreynosin (XVI), m.p. 190–193°; i.r. bands (nujol): 3442, 1720, 1680, 930, and 762 cm^{-1}. (Found: C, 71·89; H, 9·04. $C_{15}H_{22}O_3$ required: C, 72·00; H, 8·80.)

Tetrahydrosantamarine (XIV)

A solution of IV in MeOH was hydrogenated by the standard procedure using Pd-C as catalyst. The product (XIV), upon recrystallization from isopropyl ether–acetone, had a m.p. of 169° (literatur evalue:[7] 164–166°). The material was identical by i.r. with dihydrobalchanin.*

Material "x" (Mixed Crystals of XIV and XV)

A solution of 50 mg of III in 15 ml of MeOH was hydrogenated with 5 mg of 5% Pd-C as catalyst (the catalyst had been prehydrogenated for 20 min). The first mole equivalent (5 ml) of hydrogen was absorbed within 15 min, while uptake of a second 5 ml of hydrogen required 15 hr. The reaction solution yielded a tetrahydroreynosin material "x", m.p. 165–169°, which was quite similar by NMR and m.p. and mixed m.p. with tetrahydrosantamarine (XIV). However, the material "x" was different from XIV in the fingerprint region of its i.r. spectrum. Material identical in all respects with "x" was obtained when a 2:1 mixture of XIV and XV was prepared and recrystallized.

Epoxysantamarine (V) from IV

A solution of 120 mg of IV in 1 ml of $CHCl_3$ was added to a solution of 220 mg of monoperphthalic acid in 30 ml of anhyd. ether. The resultant solution was heated at 36° for 5 min and then allowed to stand at 25° for 5 hr. Next, 20 ml of water and 2 g of $NaHCO_3$ were added with shaking. The $CHCl_3$ layer was removed, dried over anhyd. Na_2SO_4 and taken to dryness *in vacuo* leaving a white solid residue. Two recrystallizations of the crude material from EtOAc yielded material, m.p. 243–245°, which was identical with the natural product (V) by mixed m.p. and NMR.

Epoxysantamarine Acetate (XVII)

A sample of V was acetylated by standard procedures to yield, after recrystallization from 95% EtOH, XVII; m.p. 185–186°; i.r. bands (nujol): 1750, 1725, 1250, and 960 cm^{-1}. (Found: C, 66·48; H, 7·24; O, 26·25. $C_{17}H_{22}O_5$ required: C, 66·75; H, 7·19; O, 26·15.)

Tamaulipin-B (II)

Recrystallization of crude (II) from $CHCl_3$–petroleum ether gave pure II, m.p. 140–142°, $[\alpha]_D^{25}$ + 99·0° (*c.* 0·987, MeOH), λ_{max} (MeOH): 207 nm (ϵ 19,400); i.r. bands (nujol): 3500, 1745, and 1660 cm^{-1}. (Found: C, 72·45; H, 8·26; O, 19·52 (M.W. by mass spec., 248). $C_{15}H_{20}O_3$ required: C, 72·55; H, 8·12; O, 19·33 (M.W. 248.)

[17] C. DJERASSI, R. R. ENGLE and A. BOWERS, *J. Org. Chem.* **21**, 1548 (1956).

Tamaulipin-B Acetate (XVIII)

Acetylation of II under standard conditions yielded, after recrystallization from ether, XIX; m.p. 155–156°, $[\alpha]_D^{25}$ + 128° (*c.* 0·54, MeOH), λ_{max} (MeOH): 209 nm (ϵ 14,250); i.r. (nujol): 1745 and 1660 cm^{-1}. (Found: C, 70·28; H, 7·68; O, 22·08. $C_{17}H_{22}O_4$ required: C, 70·32; H, 7·64; O, 22·04.)

Dihydrotamaulipin-B Acetate (XX)

A solution of II was hydrogenated by standard procedures[3] to yield, after recrystallization from benzene, XIX, m.p. 73–80°. This material was acetylated in the usual way to yield, after recrystallization from $CHCl_3$–petroleum ether (1:4), XX; m.p. 139–140°, $[\alpha]_D^{25}$ + 136·0° (*c.* 0·61, MeOH); λ_{max} (MeOH): 205 nm (ϵ 9500); i.r. bands (nujol): 1760, 1740, 1660, and 1238 cm^{-1}. (Found: C, 69·81; H, 8·18; O, 21·89. $C_{15}H_{24}O_4$ required: C, 69·83; H, 8·27; O, 21·90.)

Pyrolysis of XX to an Equilibrium Mixture of XX and the Cope Product (XXI)

Under water-pump vacuum 100 mg of XX were heated at 220° for 6 min. NMR analysis of the crude reaction mixture indicated the presence of XX and XXI in a 2:3 ratio. Separation was effected by preparative TLC (silica gel G, $CHCl_3$). A less polar band yielded 43 mg of XXI, m.p. 102–104° (from ether–petroleum ether); $[\alpha]_D^{25}$ + 24° (*c.* 0·33, MeOH); i.r. (nujol): 1765, 1748, 1665, and 1230 cm^{-1}. (Found: C, 69·66; H, 8·40. $C_{17}H_{24}O_4$ required: C, 69·83; H, 8·27.)

Keto-acid (XXII) from XXI

A sample of XXI was converted to the keto-acid (XXII) by previously described procedures:[4] Ozonolysis of XXI at –40° in EtAOc followed by $KMnO_4$ oxidation in aq. dioxan gave the keto-acid (XXII), $C_{13}H_{18}O_5$, m.p. 167–169°, identical in all respects with an authentic sample.[3]

Determination of the Configuration at C-3 in Tamaulipin-B (II) by the Horeau Method[3,15]

465 mg of racemic α-phenylbutyric anhydride (1·5 mmoles) and 124 mg (0·5 mmole) of tamaulipin-B (II) were dissolved in 2·5 ml of pyridine and the mixture was allowed to stand overnight at room temperature. The reaction mixture was worked up as previously described.[15] The NMR of the EtOAc extractable material (212 mg) indicated that the product was totally esterified. The aq. $NaHCO_3$ extract yielded 320 mg of α-phenylbutyric acid, $[\alpha]_D^{25}$ + 14·8°. For a 100% optical yield the recovered acid would have shown $[\alpha]_D^{24}$ + 19·3°, therefore the optical yield is 77% in dextrorotatory acid.[15]

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