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THE STRUCTURE OF TAMAULIPIN-A

A NEW GERMACRANOLIDE FROM *AMBROSIA CONFERTIFLORA* DC. (COMPOSITAE)

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Abstract—The structure and stereochemistry of tamaulipin-A (Ia), a new germacranolide isolated from *Ambrosia confertiflora*, was determined, in part, by conversion to a product also derived from saussurea lactone, a sesquiterpene lactone of known absolute structure.

INTRODUCTION

WE PREVIOUSLY described two new pseudoguaianolides, confertiflorin and desacetyl-confertiflorin, from central Texas populations of the common North American ragweed *Ambrosia confertiflora* DC.[1] and, in connection with our investigation of the infraspecific variation of sesquiterpene lactones in *Ambrosia* species,[2] it was of interest to study the chemistry of Mexican populations of *A. confertiflora*. This paper describes the isolation and structure determination of a new germacranolide (a sesquiterpene lactone containing a 10-membered carbocyclic unit) from certain Mexican populations of *A. confertiflora*.

Isolation and physical properties of tamaulipin-A

Collections of *A. confertiflora* from the Mexican state of Tamaulipas near Cuidad Victoria afforded a sesquiterpenoid-containing syrup in about 3% yield from the dry plant material. The two major constituents, which belonged to the germacranolide class of sesquiterpene lactones, were named tamaulipin-A and -B. In addition to tamaulipin-A and -B,[3] two eudesmanolide-type sesquiterpene lactones were isolated and their structures are presently under investigation.

Tamaulipin-A (Ia), $C_{15}H_{20}O_3$, m.p. 159–160°, $[\alpha]_D$ +171°, was present in approximately 0·5% by weight of the dry plant material. The UV spectrum (λ_{max} 208 nm, ε 19,100) of tamaulipin-A was in accord with the presence of an α,β'-unsaturated γ-lactone and a *trans*-annular conjugated 1,5-diene typical of germacranolides such as costunolide[4] (VI). IR bands indicated the presence of OH (3480), α,β'-unsaturated γ-lactone (1745) and double bonds (1660 cm^{-1}) in tamaulipin-A.

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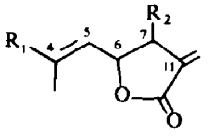
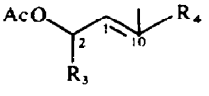
The 100 Mc NMR spectrum of tamaulipin-A acetate (Ib, $C_{17}H_{22}O_4$, m.p. 114–115°) exhibited signals typical for two vinyl Me groups, each of which was coupled to a vinyl proton (1·79, d, J = 1·2; 1·58, d, J = 1)* an acetate Me (2·06); C-6 lactonic proton (4·57, dd, J = 8·5 and 10); two vinyl protons (two overlapping broadened doublets, J about 10 for each, centered at 5·02); a proton attached to a C atom bearing an AcO function (5·69, dt, J = 5·5 and 10), and a pair of doublets for two C-11 methylene protons (5·58, d, J = 3; 6·3, d, J = 3·6). Spin-decoupling experiments suggested structure Ib (without stereochemistry) for tamaulipin-A acetate: the C-7 proton could be ascribed to complex signals near 2·6 since irradiation at a chemical shift corresponding to either of the C-11 methylene protons did not affect the signal for the other C-11 proton but did alter the 2·6 region. Moreover, irradiation at 4·57 (C-6 lactonic proton) also altered the 2·6 region and, in addition, affected the vinyl proton signal centered at 5·02. Irradiation of the vinyl proton region (5·02) collapsed, as expected, the C-6 lactonic double doublet to a doublet (J = 10) and at the same time transformed both of the vinyl Me group doublets into singlets and simplified the signals for the proton attached to the C atom bearing the AcO function. Thus, the data suggested the presence of the two fragments m and n in tamaulipin-A acetate.



m



n

That R_1 in m corresponded to R_3 in n and represented a CH_2 unit was suggested by performing spin-decoupling experiments with the signal assigned to one of the C-3 protons: a one-proton double doublet centered at 2·7 (J = 5·5 and 11). The double doublet at 2·7 collapsed to a doublet (J = 11) when the acetate proton region (5·69) was irradiated (the vinyl proton region at 5·02 was also affected), thus suggesting that one of the C-3 protons (designated H-3a) was coupled to only two protons, H-3b and the C-2 acetate proton. The signal for H-3b overlapped other aliphatic proton signals around 2·3. Finally, irradiation at the 2·6–2·7 region of the spectrum (C-7 proton and H-3a) collapsed the C-11 methylene proton doublets into singlets and the complex acetate proton signal to a double doublet (J = about 10 for each coupling). Structure Ib represents the only arrangement of the functional groups in a 10-membered carbocyclic ring which would produce the spin-decoupling data described above.

Dihydrotamaulipin-A. Tamaulipin-A (Ia) was smoothly hydrogenated to a dihydro derivative (IIa) with Pd–C as catalyst. Although most pseudoguaianolide-type sesquiterpene lactones give a mixture of the dihydro and iso (in which the C-11 exocyclic double bond has migrated into the lactone ring) products,[1] tamaulipin-A

* All chemical shift values are reported in ppm (δ-scale); coupling constants (J values) are in c/s.

gave quantitatively only the dihydro derivative.* It was of interest to note that treatment of dihydrotamaulipin-A with acetic acid–acetic anhydride did not cause cyclization to the eudesmanolide-type sesquiterpene lactones in contrast to the results reported for costunolide (VI) and related compounds.[5]

The Cope product from dihydrotamaulipin-A. Pyrolysis of dihydrotamaulipin-A for 5 min at 185° produced quantitatively the aldehyde IIIa, $C_{15}H_{22}O_3$, m.p. 101–102°, IR; 1767 (γ-lactone), 1718 (aldehyde) and 1640 cm^{-1} (double bond). The NMR spectrum of IIIa (Table 1) exhibited a characteristic triplet ($J = 3$) at 9·82 for the aldehydic proton. Oxidation of the aldehyde group in IIIa with periodate–permanganate reagent[6]† produced an acid IIIb without additional oxidation and cleavage of the double bond. The conversion of dihydrotamaulipin-A to the aldehyde IIIa‡ and the acid IIIb provided further support for the location of the functional groups as shown in IIa, particularly with respect to positioning the OH function at C-2.

Conversion of dihydrotamaulipin-A acetate to 2-acetoxysaussurea lactone. The final proof of the structure and stereochemistry of dihydrotamaulipin-A acetate, and thus tamaulipin-A, was provided by the conversion of IIb to the acid Vb, a product also obtained by ozonolysis and oxidation of saussurea lactone (IVa), a sesquiterpene lactone of known absolute structure.[10,15]

Pyrolysis of dihydrotamaulipin-A acetate for 4 min at 220° gave a 1:1 mixture§ of starting material and 2-acetoxysaussurea lactone [IVb, $C_{17}H_{24}O_4$, m.p. 138–141°; IR: 1760 (γ-lactone), 1770 and 1230 (enol acetate) and 1670 cm^{-1} (double bonds)]. The NMR spectrum of IVb (Table 1) exhibited an acetate Me singlet at 2·11 and doublets at 5·49 and 7·05 for the C-1 and C-2 vinyl protons, respectively. The coupling constant ($J = 13$) for the interaction of the C-1 and C-2 vinyl protons was in accord with a *trans* arrangement of the protons. For example, in other enol esters the olefinic protons show a *trans* coupling of about 12–14 c/s while the *cis* coupling is 7 c/s or less.[11]

The 2-acetoxysaussurea lactone (IVb) was ozonized to the keto-aldehyde Va, which was oxidized with slightly acidic permanganate to the keto-acid Vb, $C_{13}H_{18}O_5$, m.p. 167–169°; IR 1774 (γ-lactone), 1736 (carboxyl) and 1712 cm^{-1} (keto). The keto-acid Vb obtained from dihydrotamaulipin-A acetate was identical by NMR and IR spectra and m.p. and mixed m.p. with the product derived by ozonolysis and oxidation of saussurea lactone (IVa).

The evidence presented above provided structure IIb for dihydrotamaulipin-A acetate with the exception of the stereochemistry at C-2. We propose that the C-2

* The difference in products from the hydrogenation of *Ambrosia* pseudoguaianolides and germacranolides using Pd–C as catalyst presumably occurs because in most pseudoguaianolides steric factors require that the molecules rest upon the catalyst such that the C-7 alpha proton is in direct contact with the catalyst and can thus be abstracted; in contrast, in most germacranolides the stereochemistry favors hydrogenation such that the C-7 alpha proton is oriented away from the catalyst. This is in agreement with the observation that hydrogenation of pseudoguaianolides produces a beta-oriented C-11 methyl group[1] while germacranolides yield an alpha-oriented C-11 methyl.

† Dr. von Rudloff[6] has suggested privately that when the periodate–permanganate reagent is used in dioxan–water (as in this instance) rather than, for example, tertiary butyl alcohol, it is less reactive towards double bonds.

‡ Similar Cope rearrangements have been referred to as "oxy-Cope rearrangements" or "enologenic Cope rearrangement". However, we agree with A. Viola[9] that the expression "Cope rearrangements" includes those reactions which produce enols which isomerize to carbonyl functions.

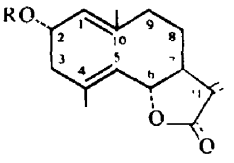
§ The products appear to represent an equilibrium mixture. We are presently studying the scope of these equilibrium reactions.

TABLE 1. NMR-DATA OF TAMAULIPIN-A AND DERIVATIVES[a]

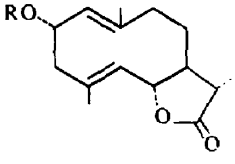
Compound	H_1 H_5	H_2	H_6	C_4 and C_{10} methyls	C_{11}—CH_2	C_{11}—CH_3	Misc.
Ia	5·00 brd (10)	4·70 dtr d (5·5) tr (10·5)	4·56 dd (8·5) (10·0)	1·74 d (1·2) 1·49 d (1·0)	6·30 d (3·8) 5·58 d (3·2)		
Ib	5·02 brd (10)	5·69 dtr d (5·5) tr (10·0)	4·57 dd (8·5) (10·0)	1·79 d (1·2) 1·58 d (1·0)	6·30 d (3·6) 5·58 d (3·6)		2·06 (Ac)
IIa	4·9 c	4·60 c	4·55 c	1·72 d (1·1) 1·48 br		1·24 d (6·5)	
IIb	4·91 br	5·63 dtr d (5·5) tr (10·0)	4·54 dd (7·5) (10·0)	1·75 br 1·56 br		1·22 d (7·0)	2·03 (Ac)
IIIa	H_1 (2H) 2·47 d (3·0) H_5 2·47 d (12·0)	9·82 tr (3·0)	4·11 brdd (12·0) (10·0)	C_4 1·84 brs C_{10} 1·10		1·22 d (7·0)	4·82 br 5·13 br } $2H_3$
IIIb	H_1 2·42 (2H) H_5 2·62 d (11·5)	8·0 br	4·11 brdd	C_4 1·84 brs C_{10} 1·10		1·23 d (7·0)	4·87 br 5·12 br } $2H_3$
IVb	H_1 (1H) 5·49 d (13·0) H_5 (1H) 2·23 d (10)	7·05 d (13·0)	4·11 brdd	C_4 1·78 br C_{10} 1·12		1·22 d (6·5)	4·72 br 5·06 br } $2H_3$ 2·11 (Ac)
Va	H_1 9·85 (—CHO) H_5 3·09 d (11·5)		4·2 br	C_4 2·27 C_{10} 1·23		1·25 d (6·5)	
Vb	H_5 3·26 d (12·0)		4·15 br	C_4 2·28 C_{10} 1·34		1·25 d (6·5)	

[a] Spectra were determined in $CDCl_3$ on a Varian A-60 spectrometer. Values are given in ppm relative to TMS as an internal standard. Numbers in parentheses denote coupling constants in c/s. Singlets are unmarked, multiplets are described as follows (or combinations thereof): d = doublet, tr = triplet, c = complex signal whose center is given, br = broad, brs = broadened singlet.

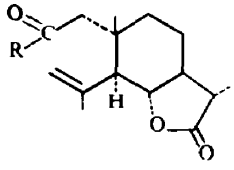
acetate function in IIb has an alpha orientation for the following reasons. The Cope rearrangement in 1,5-dienes has been shown to proceed through a quasi chair transition state.[12,13] The stereochemistry of the two newly formed asymmetric centers (C_5 and C_{10}) in IVb indicates that, in agreement with the above findings, the conversion of dihydrotamaulipin-A acetate (IIb) to 2-acetoxysaussurea lactone (IVb) also involves a chair-like transition state, and from such a conformation only an alpha oriented C-2 acetoxy-group in IIb would give rise to the previously mentioned transarrangement of the C-1 and C-2 olefinic protons in IVb.*



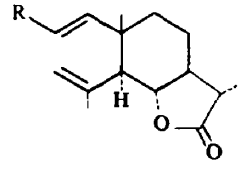
Ia: R = H
b: R = Ac



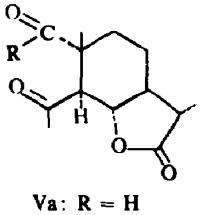
IIa: R = H
b: R = Ac



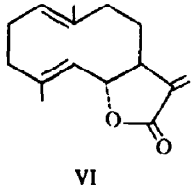
IIIa: R = H
b: R = OH



IVa: R = H
b: R = OAc



Va: R = H
b: R = OH



VI

On the basis of structure IIb for dihydrotamaulipin-A acetate, structure Ia follows for tamaulipin-A.

EXPERIMENTAL

M.ps are uncorrected. Analyses were determined by Dr. Alfred Bernhardt, Max-Planck Institut für Kohlenforschung, Mülheim, West Germany.

Isolation of tamaulipin-A. Collections of *Ambrosia confertiflora* DC. were made on 11 July 1966 (voucher No. 249619),† and 4 May 1967 (voucher No. 255649) 16 and 100 miles, respectively, north of Cuidad Victoria, Mexico.

Air-dried, ground leaves were extracted with $CHCl_3$ and worked up in the usual manner.[14] From 500 g of plant material were obtained 29 g of a thick syrup. NMR analysis indicated the presence of four new

* This assignment is based on the assumption that a thermal isomerization of the enolacetate function in IVb did not occur under the reaction conditions.

† All voucher specimens are deposited in the University of Texas Herbarium, Austin.

sesquiterpene lactones. The two major compounds were present in a 1:1 ratio; the concentration of two minor constituents (also in a 1:1 ratio) was about ¼ that of the major constituents. The crude syrup was chromatographed over silica gel, using ether as the eluting solvent. The first fractions, which gave a dark brown syrup, were followed by fractions containing one of the major substances, which we named tamaulipin-B. A number of subsequent fractions yielded mixtures of tamaulipin-B and the two minor substances in various ratios. Finally, the second major compound, tamaulipin-A, was obtained. The fractions containing pure tamaulipin-A crystallized on standing overnight at room temp.

A final yield of about 2·5 g each of crystalline tamaulipin-A and -B was obtained. In addition, about 250 mg of a 1:1 crystalline mixture of the two minor substances was isolated. Tamaulipin-A (Ia) was recrystallized from $CHCl_3$:ether, 1:2; m.p. 159–160°,* $[\alpha]_D$ +171·0° (MeOH, c = 1·08); UV (MeOH): 208 nm (ε 19,100); IR (Nujol): bands at 3480 (OH), 1745 (α,β′-unsaturated γ-lactone), 1660 cm^{-1} (double bonds). [Found: C, 72·61; H, 8·10; O, 19·23. Mol. wt. (mass spec.) 248. $C_{15}H_{20}O_3$ requires: C, 72·55; H, 8·12; O, 1933%. Mol. wt. 248].

Acetate of tamaulipin-A (Ib). A soln of 50 mg of Ia in 1 ml pyridine and 0·5 ml of Ac_2O was left overnight at room temp. Evaporation under reduced press gave a crystalline residue. Recrystallization from ether yielded 45 mg of Ib, m.p. 114–115°, $[\alpha]_D$ +143° (MeOH, c = 0·452); UV (MeOH): 206 nm (ε 19,300); IR (Nujol): bands at 1770 (lactone), 1735 (acetate) and 1665 cm^{-1} (double bonds). [Found: C, 70·37; H, 7·70; O, 22·13. Mol. wt. (mass spec.) 290. $C_{17}H_{22}O_4$ requires: C, 70·32; H, 7·64; O, 22·04%. Mol. wt. 290].

Hydrogenation of tamaulipin-A. 5% Pd–C catalyst (50 mg) in 15 ml MeOH was pre-hydrogenated with vigorous stirring for 10 min. Addition of Ia (300 mg) to the reaction mixture initiated a rapid uptake of H_2. After one equiv of H_2 had been consumed (5 min), the reaction was interrupted (longer hydrogenation times produced a mixture of products). The catalyst was filtered off and the solvent evaporated to give a colorless syrup which solidified on trituration with ether. Recrystallization from ether yielded IIa containing 0·5 moles of ether solvate. Drying of the powdered crystals for several hr at 80° gave pure IIa, m.p. 119–120°; IR (Nujol): bands at 3450 (OH), 1765 (lactone), and 1665 cm^{-1} (double bonds).

The material was converted to an acetate for characterization. Treatment of IIa (160 mg) with Ac_2O in pyridine afforded 152 mg of crystalline material. Recrystallization from ether gave pure IIb, m.p. 140°, $[\alpha]_D$ +133° (MeOH, c = 0·85); UV (MeOH): 206 nm (ε 10,300); IR bands at 1780 (γ-lactone), 1730 and 1250 (acetate), and 1665 cm^{-1} (double bonds). [Found: C, 69·79; H, 8·24; O, 22·03. $C_{17}H_{24}O_4$ requires: C, 69·83; H, 8·27; O, 21·90%].

Treatment of dihydrotamaulipin-A with AcOH–Ac_2O. A soln of 50 mg of IIa in 1 ml glacial AcOH and 0·5 ml Ac_2O was heated on a steam bath for 1 hr. Evaporation of the solvent afforded colorless crystals which were identical with IIb by mixed m.p. and NMR analysis.

Pyrolysis of dihydrotamaulipin-A. Dihydrotamaulipin-A (50 mg) was heated in a N_2 atm at 185° in a sublimation tube. After 5 min the mixture was quickly cooled whereupon the syrup solidified. Recrystallization from $CHCl_3$/petrol gave pure IIIa, m.p. 101–102°; IR (Nujol): bands at 1767 (γ-lactone), 1718 (aldehyde) and 1640 cm^{-1} (double bond). [Found: C, 72·06; H, 8·88; O, 19·03. $C_{15}H_{22}O_3$ requires; C, 71·97; H, 8·85; O, 19·17%].

$KMnO_4$–$NaIO_4$ *oxidation of* IIIa. A soln of 50 mg of IIIa in 1 ml dioxan was diluted with a soln of 15 mg K_2CO_3 in 1 ml H_2O. During a period of 10 min a solution of 1 g $NaIO_4$ and 17 mg $KMnO_4$ in 8 ml H_2O was added until the soln remained permanganate colored. The reaction mixture was acidified with dil HCl and extracted with ether. Evaporation of the dried ($MgSO_4$) ether extract yielded 37 mg of crystalline IIIb, m.p. 208–210°.

Pyrolysis of dihydrotamaulipin-A acetate. Under water pump vacuum 200 mg of IIb was heated to 220° for 4 min. After quickly cooling, the syrup was dissolved in about 1·5 ml of warm EtOH. When the soln was left in the refrigerator for 30 min, 60 mg of crude, crystalline IVb was obtained. Recrystallization of the crude material from ether at −10° gave colorless needles, m.p. 138–141°; IR (Nujol): bands at 1760 (γ-lactone), 1770 and 1230 (acetate), and 1670 cm^{-1} (double bonds). [Found: C, 69·70; H, 8·03; O, 22·10. $C_{17}H_{24}O_4$ requires: C, 69·83; H, 8·27; O, 21·90%].

Ozonolysis and oxidation of IVb. Compd IVb (50 ml) in 5 ml of EtOAc was ozonized at −45° for 30 min. The excess of O_3 was removed with a stream of N_2, and the cold reaction mixture was hydrogenated over 5% Pd–C (20 mg) for 1 hr. After filtering off the catalyst the solvent was evaporated to give 53 mg of crude material. Distillation of the material in a glass tube at 150°/0·05 torr yielded 28 mg of a colorless

* The m.p. was obtained by placing crystals on a hot plate at different temperatures; under normal procedures the substance polymerized.

syrup which was shown to be mainly compound Va by NMR analysis. Without further purification Va was converted to Vb. The above syrup was dissolved in 1 ml of dioxan and diluted with 2 ml of water. Under stirring a small excess of a slightly acidic aqueous soln of $KMnO_4$ was added dropwise to the mixture. After filtering off the MnO_2 the filtrate was extracted with $CHCl_3$; the latter soln was extracted with 5% $NaHCO_3$ aq. The alkaline extract was acidified with 2N HCl and re-extracted with $CHCl_3$. The organic layer afforded (after drying, $MgSO_4$) 12·5 mg of crystalline material. Recrystallization of the crude product from $CHCl_3$/petrol yielded crystals, m.p. 164–168°. The material was identical with Vb by mixed m.p., IR and NMR analyses.

Ozonolysis and oxidation of saussurea lactone (IVa). Compd IVa (m.p. 146–148°; 90 mg) in 10 ml of EtOAc was ozonized with excess O_3 at −70°. The excess O_3 was removed with N_2 and the reaction mixture was subsequently hydrogenated for 1 hr with 30 mg 5% Pd–C as catalyst. The catalyst was filtered off and the solvent was removed *in vacuo* to give 100 mg of crude product. Distillation of 50 mg of the syrup in a sublimation tube at 150°/0·5 torr afforded 25 mg of a colorless syrup. The NMR spectrum of this substance was in agreement with structure Va. Compound Va (25 mg) was oxidized with permanganate by the procedure described above: yield 18 mg crystalline material. Recrystallization of the crude product from ether/petrol gave colorless crystals, m.p. 163–165°. The pure substance, m.p. 167–169°, showed IR bands (KBr) at 1774 (γ-lactone), 1736 (carboxyl) and 1712 cm^{-1} (keto). [Found: C, 61·63; H, 7·31. $C_{13}H_{18}O_5$ requires: C, 61·45; H, 7·09%].

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