Phytochemistry, 1970, Vol. 9, pp. 1611 to 1614. Pergamon Press. Printed in England.

CANAMBRIN, A NEW SESQUITERPENE DILACTONE FROM *AMBROSIA CANESCENS**

J. ROMO and L. RODRÍGUEZ-HAHN

Instituto de Química de la Universidad Nacional Autónoma de México, México 20, D.F., México

(*Received* 16 *October* 1969, *in revised form* 5 *December* 1969)

Abstract—The structure of canambrin (I), a constituent of *Ambrosia canescens*, has been established as a sesquiterpene dilactone of the psilostachyin series.

IN THIS communication we report the isolation and structure determination of canambrin (I), a new sesquiterpene dilactone stereoisomeric with psilostachyin (II).[1,2] Ethanol extraction of the aereal part of *Ambrosia canescens* (Gray) Payne gave, after chromatography, a crystalline product ($C_{15}H_{20}O_5$), m.p. 209–210°; $[\alpha]_D$–134° which we propose to name canambrin.

The i.r. spectrum of canambrin (I) showed a hydroxyl band at 3600 cm^{-1}. The anomalous strength of a carbonyl band at 1770 cm^{-1} suggested the presence of two five-membered lactone functions, one being conjugated with an exocyclic methylene group, since canambrin had absorption at 212 nm (ϵ, 9900) in the u.v. spectrum and it showed a weak i.r. band at 1660 cm^{-1}. Isomerization with palladium on charcoal in a hydrogen atmosphere gave isocanambrin (III) (λ_{max} 224 nm; ϵ, 12,400).

The NMR spectrum of canambrin (I)‡ exhibited a doublet ($J = 7$ Hz) at 1·20 corresponding to a secondary methyl group. A singlet at 1·28 was assigned to a methyl group attached at a carbon atom bearing a hydroxyl group. The exocyclic methylene protons were responsible for a pair of doublets ($J = 3{\cdot}5$ Hz) at 5·58 and 6·28. A doublet ($J = 10$ Hz) at 4·73 is attributed to the C-6 hydrogen.

The NMR spectrum of isocanambrin (III) did not show the doublets corresponding to the exocyclic methylene; instead, a doublet ($J = 2$ Hz) present at 1·86 was ascribed to a vinylic methyl group.

As in psilostachyin (II),[2] $NaBH_4$ reduction of canambrin (I) gave two products. One was a dihydro derivative (IV). The i.r. spectrum showed a hydroxyl band at 3600 cm^{-1} and a carbonyl band (double strength) at 1765 cm^{-1} corresponding to the γ-lactones. The NMR spectrum exhibited doublets ($J = 7$ Hz) at 1·12 and at 1·16, ascribed to two secondary methyl groups. A singlet at 1·38 was assigned to a methyl group attached at a carbon carrying a hydroxyl group. A doublet ($J = 7$ Hz) at 4·61 was assigned to the C-6 proton.

The other product isolated from the mother liquors of dihydrocanambrin (IV) was the triol (V), as shown by the following evidence. The i.r. spectrum showed a strong hydroxyl

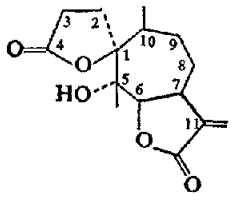
* Contribution No. 281 from the Instituto de Química de la Universidad Nacional Autónoma de México.

† We are indebted to Miss Silvia del Amo for the collection and identification of the plant.

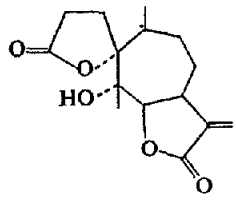
‡ NMR spectra were measured by Mr. E. Díaz in $CDCl_3$ solution with TMS as internal standard. All chemical shifts are given in ppm as δ values.

[1] H. E. MILLER, H. B. KAGAN, W. RENOLD and T. J. MABRY, *Tetrahedron Letters* 3397 (1965).
[2] T. J. MABRY, H. E. MILLER, H. B. KAGAN and W. RENOLD, *Tetrahedron* 1139 (1966).

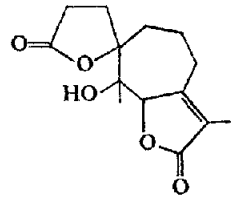
band at 3450 cm^{-1} and a γ-lactone band at 1740 cm^{-1}. Cleavage of the triol (V) in methanol solution with periodic acid afforded the derivative (VI) ($C_{16}H_{26}O_5$). The i.r. spectrum did not show hydroxyl absorption. Carbonyl bands at 1770 cm^{-1} and at 1715 cm^{-1} corresponded to a γ-lactone and a ketone, respectively. The NMR spectrum exhibited a doublet (6 H, $J = 7$ Hz) at 0·92 attributed to two secondary methyl groups. Two singlets at 2·28 and at



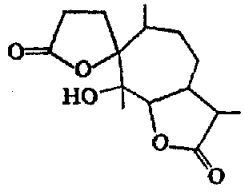
Canambrin (I)



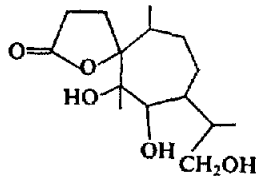
Psilostachyin (II)



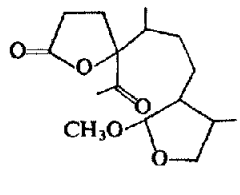
Isocanambrin (III)



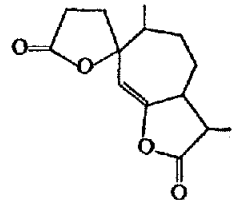
(IV)



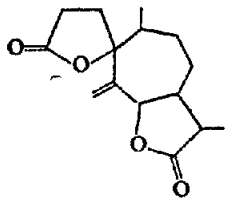
(V)



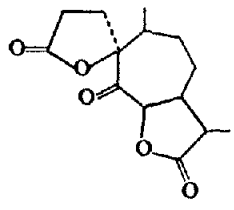
(VI)



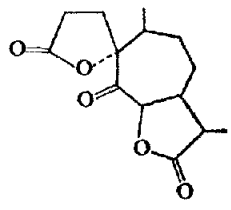
(VII)



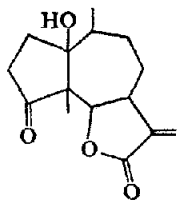
(VIII)



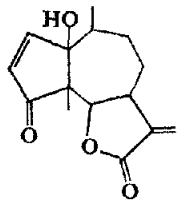
(IX)



(X)



(XI)



(XII)

3·36 were assigned to a methyl ketone and a methoxyl group, respectively. Two multiplets at 3·50 and 4·00 (1 H, each) were ascribed to the methylene group attached at the ether function. A doublet ($J = 2·5$ Hz) at 4·70 correspond to the C-6 proton.

Treatment of dihydrocanambrin (IV) with thionyl chloride in pyridine gave two products which were separated by chromatography. The anhydro derivative (VII) had, in the i.r. spectrum, a carbonyl band at 1780 cm^{-1} (double strength, γ-lactone and enol γ-lactone), and at 1690 cm^{-1} (strong, C=C double bond). The NMR spectrum showed doublets ($J = 7$ Hz)

at 1·10 and 1·23 corresponding to two secondary methyl groups. A doublet ($J = 1$ Hz) at 1·77 was assigned to a vinylic methyl group.

The isomeric anhydro derivative (VIII) had a carbonyl band at 1770 cm^{-1} (double strength, γ-lactone groups). The NMR spectrum exhibited two doublets ($J = 7$ Hz) at 0·98 and 1·16, ascribed to two secondary methyl groups. A pair of split doublets ($J = 2$ Hz) at 5·44 and at 5·61 were assigned to the exocyclic methylene protons. The multiplicity of a signal at 5·10, attributed to the C-6 proton, indicates homoallylic coupling with the exocyclic methylene.

Ozonolysis of the anhydro derivative (VIII) gave the ketone (IX). The newly formed keto group showed absorption at 1740 cm^{-1} in the i.r. spectrum. A doublet ($J = 10$ Hz) at 5·44, present in the NMR spectrum of IX, corresponded to the C-6 hydrogen.

The evidence so far described is fully in accord with structure I for canambrin. The mass spectra* of canambrin (I) and of psilostachyin (II) showed a molecular ion at 280 *m/e* and the same fragmentation pattern indicating the close relationship of both lactones. The ketone (IX) was epimerized by treatment with zinc in acetic acid. The resulting product was identified with the ketone (X) previously obtained as a degradation product in the psilostachyin series.[2],†

Though the configuration at C-5 and C-6 was not established, biogenetic considerations suggest that canambrin (I) and psilostachyin (II) differs only in the orientation of the spiranic lactone, since all the pseudoguaianolides and psilostachyins isolated from the genus *Ambrosia* possess the same stereochemistry at C-5, C-6, C-7 and C-10. The biogenetic precursor of canambrin (I) appears to be 1-epicoronopilin (XI) an unknown *cis*-fused pseudoguaianolide. However, the closely related hymenin (XII) has been very recently isolated from *Hymenoclea salsola*.[3]

EXPERIMENTAL

Melting points are uncorrected. I.r. spectra and rotations were run in $CHCl_3$ unless otherwise stated. U.v. spectra were determined in 95 per cent ethanol. The chromatograms were run on alumina Alcoa F-20 (washed with EtOAc). The analyses were performed by Dr. Franz Pascher, Bonn, Germany.

Isolation of Canambrin (I)

Ambrosia canescens was collected in August 1967 in the neighbourhood of the city of Durango. The dried plant (without roots; 250 g) was extracted with ethanol (4 l.) for 12 hr under reflux. The extract was filtered, concentrated to 1 l. and treated with a solution of lead acetate (20 g) in water (1 l.). After standing at room temperature, the solution was filtered, diluted with water and extracted twice with $CHCl_3$. The extract was evaporated to dryness and the residue, dissolved in benzene, was chromatographed on alumina (400 g). The crystalline fractions eluted with benzene, and benzene with increasing proportions of EtOAc, were combined and recrystallized from EtOAc–isopropyl ether. This yielded canambrin (I) (2·85 g), m.p. 209–210°; $[\alpha]_D$ −134°; λ_{max} 212 nm; ϵ, 9900; i.r.; 3600 cm^{-1} (hydroxyl group), 1770 cm^{-1} (γ-lactones) and 1660 cm^{-1} (C=C double bond); NMR, 1·20 (doublet, J=7 Hz, C-10 methyl group), 1·28 (singlet, C-5 methyl group), 4·73 (doublet, J=10 Hz, C-6 proton), 5·58, 6·28 (doublets, J=3·5 Hz, CH_2=); mass spectrum of canambrin (I) gave a m.w. of 280. The peaks *m/e* 262 and 237 result from the loss of H_2O and CH_3—C≡O^+, respectively. A peak *m/e* 219 can be attributed to the loss of H_2O and CO_2. (Found: C, 64·04; H, 7·23; O, 28·51. $C_{15}H_{20}O_5$ required: C, 64·27; H, 7·19; O, 28·54%.)

A. canescens collected in August 1967 in the outskirts of Cortazar, State of Guanajuato, gave also canambrin with approximately the same yield.

Isocanambrin (III). A solution of canambrin (I) (250 mg) in EtOAc (30 ml) with 5% Pd-C (80 mg) was stirred in H_2 for 8 hr. The solution was filtered and evaporated to dryness. Repeated crystallizations of the solid residue from acetone–ether yielded 60 mg of (III), m.p. 240–242°; $[\alpha]_D$ −28·3°; λ_{max} 224 nm; ϵ, 12,400; i.r.: 3600 cm^{-1} (hydroxyl group) and 1765 cm^{-1} (γ-lactones); NMR 5·01 (broad doublet, J=2 Hz, C-6 proton),

* Mass spectra were determined by Mr. E. Cortés.

† We are grateful to Dr. T. J. Mabry for a sample of ketone (X).

[3] F. P. Toribio and T. A. Geissman, *Phytochem.* 7, 1623 (1968).

1·86 (doublet, J=2 Hz, C-11 methyl group), 1·16 (doublet, J=7 Hz, C-10 methyl group), 1·13 (singlet, C-5 methyl group). (Found: C, 63·86; H, 7·25; O, 28·65. $C_{15}H_{20}O_5$ required: C, 64·27; H, 7·19; O, 28·54%.

Sodium Borohydride Reduction of Canambrin (I)

$NaBH_4$ (750 mg) in methanol (10 ml) was added to a solution of canambrin (I) (750 mg) in methanol (25 ml) at 10–15°. After 15 min the solution was diluted with water, acidified with dil. HCl and extracted with $CHCl_3$. The organic solution was washed, dried and evaporated. Crystallization from $CHCl_3$–isopropyl ether gave dihydrocanambrin (IV) as small needles (460 mg), m.p. 212–216°. Further crystallizations from the same pair of solvents raised the m.p. to 224–225°; $[\alpha]_D$ −24·6°; i.r. 3600 cm^{-1} (hydroxyl group) and 1765 cm^{-1} (five-membered lactones); NMR 4·61 (doublet, J=7 Hz, C-6 proton), 1·48 (singlet, C-5 methyl group), 1·12, 1·16 (doublets, J=7 Hz each, C-10 and C-11 methyl groups). (Found: C, 63·59; H, 7·76; O 28·63. $C_{15}H_{22}O_5$ required: C, 63·81; H, 7·85; O, 28·34%.)

From the mother liquors hexahydrocanambrin (V) was obtained as prisms (50 mg) from acetone–isopropyl ether, m.p. 190–192°; $[\alpha]_D$ + 2·7° (diox); i.r.: (nujol) 3450 cm^{-1} (hydroxyl groups) and 1740 cm^{-1} (γ-lactone). (Found: C, 63·12; H, 9·23; O, 27·91. $C_{15}H_{26}O_5$ required: C, 62·91; H, 9·15; O, 27·94%.)

Periodic Acid Oxidation of Hexahydrocanambrin (V)

A solution of V (90 mg) in methanol (10 ml) was treated with a solution of HIO_4 (200 mg) in water (2 ml) and left at room temp. for 24 hr. Water was added and the mixture was extracted with $CHCl_3$. The organic layer was washed with aq. $NaHSO_3$, water, dried and evaporated. The residue was dissolved in benzene and passed through alumina (2 g). Crystallization from acetone–hexane yielded prisms (30 mg), m.p. 98–100°; $[\alpha]_D \pm 0°$; i.r. 1770 cm^{-1} (γ-lactone) and 1715 cm^{-1} (keto group); NMR 4·70 (doublet), J=2·5 Hz, C-6 proton), 3·50, 4·00 (multiplets O—CH_2—), 3·36 (singlet, OCH_3), 2·28 (singlet, CH_3—CO), 0·92 (doublet, 6 H, J=7 Hz, C-10 and C-11 methyl groups). (Found: C, 64·46; H, 8·91; O, 27·01. $C_{16}H_{26}O_5$ required: C, 64·40; H, 8·78; O, 26·82%.)

Dehydration of Dihydrocanambrin (IV)

A solution of dihydrocanambrin (IV) (625 mg) in pyridine (10 ml) was treated at 5° with $SOCl_2$ (1·5 ml). The mixture was left at 5° for 5 min, poured into ice and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with aq. $NaHCO_3$, water, dried and evaporated. Chromatography on alumina (30 g) gave two products. The first crystalline fractions eluted with benzene were combined and recrystallized from ether–hexane. This yielded anhydrodihydrocanambrin (VII) (25 mg), m.p. 135–136°; $[\alpha]_D$ + 89·5°; i.r.: 1780 cm^{-1} (γ-lactone and five-membered enol lactone) and 1690 cm^{-1} (enolic double bond); NMR 1·77 (doublet, J= 1 Hz, vinyl methyl group), 1·10, 1·23 (doublets, J=7 Hz, C-10 and C-11 methyl groups). (Found: C, 68·13; H, 7·77; O, 24·41. $C_{15}H_{20}O_4$ required: C, 68·16; H, 7·63; O, 24·21%.)

The polar fractions eluted with benzene were combined and recrystallized from acetone–isopropyl ether. This yielded anhydro dihydrocanambrin (VIII) (160 mg), m.p. 116–118°; $[\alpha]_D \pm 0°$; i.r.: 1770 cm^{-1} (γ-lactones), NMR 5·44, 5·61 (doublets, J=2 Hz, CH_2=), 5·10 (multiplet, C-6 proton), 0·98, 1·16 (doublets, J=7 Hz each, C-10 and C-11 methyl groups). (Found: C, 67·82; H, 7·68; O, 24·09. $C_{15}H_{20}O_4$ required: C, 68·16; H, 7·63; O, 24·21%.)

Anhydronordihydrocanambrin (IX)

A solution of VIII (140 mg) in EtOAc was ozonized at −40° for 5 min and then hydrogenated in the presence of 5% Pd-C (40 mg). The solution was filtered and evaporated to dryness. Crystallization from EtOAc gave the ketone as prisms (110 mg), m.p. 200–210°; further crystallizations raised the m.p. to 227–231°; $[\alpha]_D$ −68·8°; i.r. 1780 cm^{-1} (γ-lactones) and 1740 cm^{-1} (keto group); NMR 5·44 (doublet, J=10 Hz, C-6 proton), 1·14, 1·07 (doublets, J=7 Hz each, C-10 and C-11 methyl groups). (Found: C, 62·99; H, 6·93; O, 30·33. $C_{14}H_{18}O_5$ required: C, 63·14; H, 6·81; O, 30·04%.)

Treatment of the Ketone (IX) with Zinc

A solution of IX (80 mg) in HOAc (5 ml) was treated with Zn powder (500 mg) and heated under reflux with mechanical stirring for 4 hr. Zn (500 mg) was added and the reflux prolonged for 8 hr more. The filtered solution, diluted with water, was extracted with $CHCl_3$. The organic solution was washed with water, dried and evaporated. The residue was chromatographed on alumina. The crystalline fractions eluted with benzene were combined and recrystallized from acetone–ether. This yielded the ketone (X) (30 mg), m.p. 164–168°, identified with an authentic sample.[3] The mixed m.p. showed no depression and the i.r. spectra were superimposable.

A solution of ketone IX (15 mg) in benzene (5 ml) was heated under reflux with *p*-toluenesulfonic acid (5 mg) for 2 hr. The solution was washed with aq. $NaHCO_3$, water, dried and evaporated. The residue crystallized from EtOAc. This yielded material, m.p. 222–225°, identified as ketone IX by the standard methods.

Dihydrocanambrin (IV) (160 mg) was recovered unchanged when it was treated under the conditions described above for ketone IX.