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LIGUSTRIN, A GUAIANOLIDE ISOLATED FROM *EUPATORIUM LIGUSTRINUM* DC[1]

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Abstract—The structure of ligustrin a constituent of *Eupatorium ligustrinum* DC. has been established as a sesquiterpene lactone of the guaiane series represented by formula Ia.

RECENTLY, we have examined several collections of Eupatorium species in search of sesquiterpene lactones and we now wish to describe the chemical investigation of *Eupatorium ligustrinum* DC. A *Compositae* distributed in the northern part of Veracruz.

Chromatography of the ethanol extract of *E. ligustrinum* led to the isolation of a guaianolide which we propose to name ligustrin. The evidence cited below indicates that this sesquiterpene possesses structure Ia.

Ligustrin (Ia; $C_{15}H_{18}O_3$) m.p. 135–137°, $[\alpha]_D$ +56° contains a secondary OH group (IR band at 3580 cm^{-1}) which forms an acetate and is oxidized to a keto group. Ligustrin (Ia) possesses three double bonds one of them conjugated with a γ-lactone function (IR band at 1765 cm^{-1}) as demonstrated by the UV absorption at 208 mμ (ε, 11,500).

The exocyclic methylene group was saturated by reduction with sodium borohydride or aluminium amalgam. The resulting dihydroderivative IIa had IR bands at 3550 and 1775 cm^{-1}. It had no high absorption in the region of 210 mμ in the UV spectrum. The dihydroderivative IIa gave the acetate IIb. The NMR spectrum* of ligustrin (Ia) exhibited two low field doublets (J = 3·5 c/s) at 6·38 and 5·63 corresponding to the protons of the exocyclic methylene group conjugated with the lactone. A vinylic proton is responsible for a signal found at 5·63 superimposed on one of the doublets. A pair of singlets at 5·03 and 4·97 with apparent long range coupling are assigned to the exocyclic methylene group substituted at C-10.† A pair of doublets (J = 8·5 c/s) partially superimposed, centred at 4·42 are attributed to the proton attached to the carbon bearing the ethereal oxygen of the lactone. The above pair of doublets is superimposed on a multiplet ascribed to the proton bonded to the carbon carrying the OH group. In the NMR spectra of Ib and IIb this multiplet is shifted downfield (at 5·57 and at 5·22, respectively) whereas the signal corresponding to the proton bonded to the carbon bearing the ethereal oxygen of the lactone remains stationary and is observed as a triplet (J = 10 c/s) characteristic in the guaianolide series of a lactone oriented at C-6. In the NMR spectrum of ligustrin (Ia) a vinylic Me group is

* The NMR spectra were determined by Mr. Eduardo Díaz on a Varian A-60A spectrometer, in $CDCl_3$ soln using TMS as internal reference. All chemical shifts are reported in ppm as δ values.

† The chemical shift of these signals is very similar to that of the singlets corresponding to the exocyclic methylene group substituted at C-10 of Zaluzamin C (see Ref. 3).

responsible for a slightly broadened doublet (J = 1 c/s) at 1·85 with apparent long range coupling. The NMR spectrum of dihydroligustrin IIa did not exhibit the low field doublets corresponding to the exocyclic methylene conjugated with the lactone. A doublet (J = 7 c/s) at 1·26 is assigned to a secondary Me group. Chromium trioxide oxidation of dihydroligustrin IIa gave a dehydro derivative (III). Its UV spectrum showed a maximum at 287 mμ (ε, 110) due to the transition n → π* of the β,γ-unsaturated ketone. The latter is substituted in the 7-membered ring as shown by a CO band at 1715 cm^{-1} in the IR spectrum of dehydrodihydroligustrin (III). The NMR spectrum of the ketone III had the following signals: An unresolved multiplet (1H) at 5·59 and a complex signal (3H) which could be defined as a quadruplet (J = 2 c/s) at 1·87, both signals exhibit long range coupling (—CH=C—CH_3), two quadruplets (J = 1 c/s) at 5·23 and 5·07 showing also long range coupling (C-10 exocyclic methylene), a triplet at 4·14 (J = 10 c/s) (C-6 proton), a sharp signal at 3·30 (2H, C-9 hydrogens), a doublet (J = 7 c/s) at 1·24 (secondary Me group).

Careful acid treatment of a benzene solution of III caused isomerization of the C-10 exocyclic double bond to endocyclic conjugation. The resulting α,β-unsaturated cycloheptanone IV had a strong UV absorption at 239 mμ (ε, 11,300). IR bands at 1775 cm^{-1} (γ-lactone) and at 1660 cm^{-1} (cycloheptenone). The signals corresponding to the C-10 exocyclic methylene observed in the NMR spectrum of the ketone III are not present in the spectrum of IV. The latter exhibited a triplet (J = 1·5 c/s) at 6·01 ascribed to the vinylic proton at C-9. An unresolved multiplet at 5·61 corresponds to the vinylic hydrogen at C-3. Two vinylic Me groups are responsible for two signals partially superimposed which could be defined as a doublet (J = 1·5 c/s) at 2·00 and a triplet (J = 1 c/s) at 1·93.

A more drastic acid treatment of the ketone III resulted in formation of the unconjugated ketone V. It did not show high absorption in the UV spectrum. A carbonyl band at 1720 cm^{-1} in the IR spectrum of V corresponds to a cycloheptanone. The NMR spectrum of the ketone V showed only the vinylic proton substituted at C-3 as an unresolved multiplet at 5·52. A pair of doublets (J = 10 c/s) centered at 4·47, a triplet (J = 6·5 c/s) at 3·78 and a multiplet (J = 7 c/s) at 2·66 are attributed to the C-6, C-7 and C-11 protons, respectively. A complex signal (6H) at 1·76 is assigned to two vinylic methyls. A secondary Me group is responsible for a doublet (J = 7 c/s) at 1·28.

Hydrogenation of the ketone V afforded a tetrahydro derivative (VI). Its IR spectrum had bands at 1780 cm^{-1} (γ-lactone) and at 1710 cm^{-1} (7-membered ketone). Three doublets at 1·26, 1·08 and 0·92 are found in the NMR spectrum of VI corresponding to three secondary Me groups.

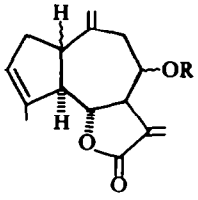
Chromium trioxide oxidation of ligustrin (Ia) confirmed the relative position of the OH group and the lactone in the 7-membered ring, already deduced from the transformations described above. It gave a cross conjugated ketone (VII; λ_{max} 262 mμ, ε, 12,700). Its IR spectrum had bands at 1770 cm^{-1} (γ-lactone), weak bands at 1665 cm^{-1} (cycloheptenone),* at 1635 and 1615 cm^{-1} (C=C double bonds). The NMR spectrum of VII showed a quadruplet (J = 1 c/s) at 6·17 and an unresolved multiplet at 5·76 ascribed to the C-9 and C-3 vinylic protons. A pair of broad doublets

* The cycloheptanone bands very frequently are weaker than those corresponding to the γ-lactone. This also occurs in the IR spectra of III, IV, V and VI.

(J = 2 c/s) with apparent long range coupling centered at 5·13 is assigned to the C-6 proton. Three vinyl Me groups are responsible for a doublet (J = 2 c/s) at 2·30, a doublet (J = 1 c/s) at 2·07 partially superimposed on a triplet (J = 1 c/s) at 2·00.

Pyrolysis of ligustrin (Ia) in the presence of Pd–C permitted the establishment of the guaiane skeleton of Ia. This reaction gave chamazulene VIII characterized as its TNB adduct.

We could obtain a further proof concerning the structure of ligustrin in the following way. Desulfurization of the cycloethylene thioketal of dehydrodihydroligustrin IX with Raney Ni occurred with simultaneous reduction of the exocyclic double bond since the crude product obtained from this reaction yielded a crystalline monoepoxide X on treatment with *m*-chloroperbenzoic acid. This epoxide (X) was identified as tetrahydroestafiatin C obtained by catalytic hydrogenation of estafiatin (XI).[2] Therefore the asymmetric centers of ligustrin at C-1, C-5, C-6 and C-7 possess the same orientation as in estafiatin (XI).[2] Treatment of the epoxide X with BF_3-etherate afforded a ketone which proved to be identical with tetrahydroestafiatone XII (obtained also as a transformation product of zaluzanin C).[3] This correlation permit to establish the stereochemistry of the C-5, C-6 and C-7 asymmetric centers of ligustrin as shown in formula Ia.



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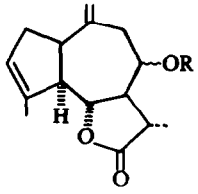
b: R = Ac



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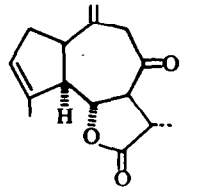

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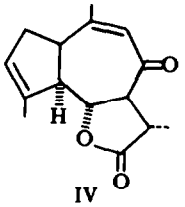

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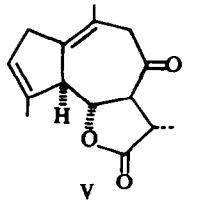

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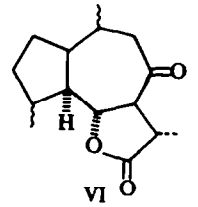

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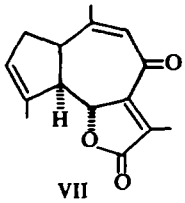

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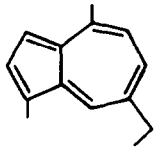

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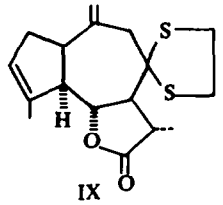

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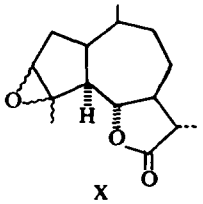

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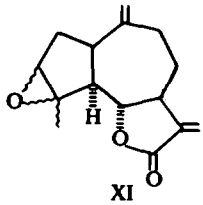

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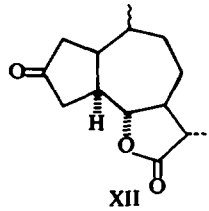

XII

EXPERIMENTAL

M.ps are uncorrected. Analyses by Dr. Franz Pascher, Bonn, Germany. UV spectra: 95% EtOH soln, Beckman DK2 spectrophotometer. IR spectra: $CHCl_3$ soln, Perkin–Elmer 21 double beam spectrophotometer. The alumina used in the chromatograms was Alcoa F-20 (washed with AcOEt). Rotations in $CHCl_3$ at 20°.

Isolation of ligustrin (Ia). *Eupatorium ligustrinum* DC was collected in October (1966) in Sierra de Chiconquiaco (Estado de Veracruz). The dried plant (8·5 Kg) was extracted with EtOH (22 l.) for 16 hr under reflux. The extract was filtered, concentrated to 3 L and treated with a soln of lead acetate (80 g) in water (3 l.), left at room temp, 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 (1 Kg). The crystalline fractions eluted with benzene were combined and recrystallized from acetone–ether. This yielded Ia (2·1 g), m.p. 135–137°; $[\alpha]_D$ +56°; λ_{max} 208 mμ; ε, 11,500; IR bands at 3580 cm^{-1} (OH group), at 1765 cm^{-1} (γ-lactone) and at 1640 cm^{-1} (C=C double bond). (Found: C, 72·97; H, 7·36; O, 19·64. Calc. for $C_{15}H_{18}O_3$: C, 73·14; H, 7·37; O, 19·49%).

Ligustrin acetate (Ib). Acetylation of Ia with Ac_2O–pyridine on the steam bath for 1 hr yielded Ib, m.p. 106–107°; $[\alpha]_D$ +22°; λ_{max} 207 mμ; ε, 10,300; IR bands at 1765 cm^{-1} (γ-lactone), at 1740 cm^{-1} (acetyl group), at 1670 and 1640 cm^{-1} (C=C double bonds). (Found: C, 70·70; H, 6·71; O, 22·63. Calc. for $C_{17}H_{20}O_4$: C, 70·81; H, 6·99; O, 22·20%).

Dihydroligustrin IIa. To a soln of Ia (400 mg) in EtOH (100 ml) was added Al amalgam (2 g) and the mixture was heated under reflux for 13 hr. The soln was filtered, evaporated to dryness *in vacuo* and the residue crystallized from acetone–isopropyl ether. This yielded IIa (325 mg) m.p. 163–164°; $[\alpha]_D$ +92°; IR bands at 3550 cm^{-1} (OH group), at 1775 cm^{-1} (γ-lactone) and at 1640 cm^{-1} (C=C double bond). (Found: C, 72·39; H, 8·21; O, 19·32. Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12; O, 19·33%).

A soln of Ia (300 mg) in MeOH (15 ml) was treated at 0° with $NaBH_4$ and stirred for 15°, acidified with AcOH and evaporated to dryness *in vacuo*. The residue dissolved in benzene was passed on alumina (4 g) and crystallized from acetone–isopropyl ether. This yielded IIa (190 mg) m.p. 162–163°.

Dihydroligustrin acetate (IIb). Acetylation of IIa with Ac_2O-pyridine for 1 hr on the steam bath afforded IIb, m.p. 108°; $[\alpha]_D$ +15°; IR bands at 1765 cm^{-1} (γ-lactone), at 1740 cm^{-1} (acetyl group) and at 1645 cm^{-1} (C=C double bond). (Found: C, 70·15; H, 7·74; O, 22·11. Calc. for $C_{17}H_{20}O_4$: C, 70·32; H, 7·64; O, 22·04%).

Chromium trioxide oxidation of ligustrin (Ia). A soln of Ia (150 mg) in acetone (6 ml) was treated with 8N CrO_3 at 5° until the persistence of an orange colour. The soln was diluted with AcOEt, washed with water, dried and evaporated to dryness. Chromatography on alumina (2 g) and crystallization from acetone–hexane afforded VII (30 mg) m.p. 170–174°; λ_{max} 201, 262 mμ; ε, 15,900, 12,700; IR bands at 1770 cm^{-1} (γ-lactone), at 1665 cm^{-1} (cycloheptenone), at 1635 and at 1615 cm^{-1} (C=C double bonds). (Found: C, 73·76; H, 6·68; O, 19·60. Calc. for $C_{15}H_{16}O_3$: C, 73·75; H, 6·60; O, 19·65%).

Dehydrodihydroligustrin (III). A soln of IIa (325 mg) in acetone (20 ml) was treated with 8N CrO_3 at 5°. Chromatography on alumina (4 g) and crystallization from acetone–hexane yielded III (180 mg) m.p. 115–118°; $[\alpha]_D$ +80°; λ_{max} 287 mμ; ε, 110; IR bands at 1780 cm^{-1} (γ-lactone), at 1715 cm^{-1} (cycloheptanone) and at 1645 cm^{-1} (C=C double bonds). (Found: C, 72·93; H, 7·39; O, 20·06. Calc. for $C_{15}H_{18}O_3$: C, 73·14; H, 7·37; O, 19·49%).

Isomerization of ketone III. A soln of III (90 mg) in benzene (6 ml) was treated with *p*-toluenesulfonic acid (40 mg) and heated under reflux for 30 min. The soln was diluted with AcOEt, washed with aq $NaHCO_3$, dried over Na_2SO_4 and evaporated to dryness. The residue crystallized from ether–isopropyl ether. This yielded IV (40 mg) m.p. 125–127°; $[\alpha]_D$ −164°; λ_{max} 204, 239 mμ; ε, 6000, 11,300; IR bands at 1775 cm^{-1} (γ-lactone), at 1660 cm^{-1} (cycloheptenone) and at 1620 cm^{-1} (C=C double bonds). (Found: C, 73·06; H, 7·46; O, 19·40. Calc. for $C_{15}H_{18}O_3$: C, 73·14; H, 7·37; O, 19·49%).

Treatment of dehydrodihydroligustrin (III) *with HCl*. A soln of III (100 mg) in MeOH (8 ml) was treated with conc HCl (0·8 ml), heated under reflux for 1 hr, concentrated *in vacuo*, diluted with water and extracted with AcOEt. The organic layer was washed with $NaHCO_3$ aq, dried over Na_2SO_4 and evaporated to dryness. The residue was passed through silica gel and crystallized from acetone–hexane. This yielded V (45 mg) m.p. 218–220°; $[\alpha]_D$ +222°; λ_{max} 207 mμ; ε, 5100; IR bands at 1780 cm^{-1} (γ-lactone) and at 1720 cm^{-1} (cycloheptanone). (Found: C, 73·20; H, 7·40; O, 19·38. Calc. for $C_{15}H_{18}O_3$: C, 73·14; H, 7·37; O, 19·49%).

Dehydrohexahydroligustrin VI. A soln of V (130 mg) in AcOEt (20 ml) was hydrogenated in the presence of 5% Pd–C (40 mg) until the absorption of H_2 ceased. The soln was filtered and evaporated to dryness. Sublimation under high vacuum of the residue yielded the analytical sample (40 mg) m.p. 175°; $[\alpha]_D$ +180°;

IR bands at 1780 cm^{-1} (γ-lactone) and at 1710 cm^{-1} (cycloheptanone). (Found: C, 71·71; H, 8·91; O, 19·43. Calc. for $C_{15}H_{22}O_3$: C, 71·97; H, 8·86; O, 19·17%).

Aromatization of ligustrin (Ia). A mixture of Ia (480 mg), nujol (4 ml) and 5% Pd–C (750 mg) was heated to 300° for 15 min, cooled, diluted with hexane filtered and chromatographed on alumina (30 g). The blue fraction was evaporated to dryness and the azulene purified through the H_3PO_4 adduct. The latter was decomposed with ice-water and extracted with hexane. The organic soln was evaporated to dryness and the residue converted to the TNB adduct. Crystallization from MeOH gave the TNB adduct of chamazulene (15 mg) m.p. 128–129°. Identified with an authentic specimen by the standard methods.

Cycloethylene thioketal of dehydrodihydroligustrin (IX). A soln of III (550 mg) in ethanedithiol (1 ml) and BF_3-etherate (1 ml) was left at room temp for 2 hr and diluted with AcOEt. The organic soln was washed with $NaHCO_3$ aq, water, dried over Na_2SO_4 and evaporated to dryness. Crystallization of the residue from acetone–hexane yielded IX (380 mg) m.p. 138–140°; $[\alpha]_D$ +58°; IR bands at 1760 cm^{-1} (γ-lactone) and at 1635 cm^{-1} (C=C double bonds). (Found: C, 63·43; H, 6·95; O, 10·01; S, 19·71. Calc. for $C_{17}H_{22}O_2S_2$: C, 63·34; H, 6·88; O, 9·92; S, 19·85%).

Epoxide X. A soln of IX (350 mg) in EtOH (80 ml) was treated with Ni–Ra (4 g), heated under reflux for 6 hr, filtered and the soln evaporated to dryness. The residue was dissolved in $CHCl_3$ (15 ml),350 mg of *m*-chloroperbenzoic acid was added and the mixture heated under reflux for 2 hr, washed with $NaHCO_3$ aq and evaporated to dryness. The residue was dissolved in hexane and chromatographed on alumina (6 g). The crystalline fractions were combined and recrystallized from ether–hexane. This yielded (70 mg) m.p. 139–140° undepressed on admixture with the tetrahydroestafiatin m.p. 144–146°.[5] The IR spectra were superimposable.

Treatment of the epoxide X *with* BF_3. A soln of X (60 mg) in benzene (6 ml) was treated with BF_3-etherate (0·5 ml) left for 1 hr at room temp and diluted with AcOEt. The organic layer was washed with $NaHCO_3$ aq, dried over Na_2SO_4 and evaporated to dryness. Crystallization of the residue from acetone–hexane yielded XII (40 mg) m.p. 197–200° undepressed on admixture with tetrahydroestafiatone.[2] The IR spectra were superimposable.

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