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STUDIES ON TETRANORTRITERPENOIDS FROM *CEDRELA AUGUSTIFOLIA* SESSÉE AND MOC[1]

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Abstract—Three new bicyclononanolides: angustinolide (I), angustidienolide (IIa) and 2α-hydroxyangustidienolide (III) isolated from *Cedrela angustifolia* have been identified. Their respective structures were determined by spectroscopic means and chemical transformations leading to known compounds. A relationship was determined between the location of the 17-H signal in the NMR and the position of the double bond in rings C and D in several related compounds.

IN THE course of a systematic investigation of the bitter principles of brazilian Meliaceae[1], *Cedrela angustifolia* Sessé and Moc. has been analysed and three new bicyclononanolides have been isolated and identified as augustinolide (I), augustidienolide (IIa) and 2α-hydroxyangustidienolide (III). Their respective structures and their relationship to known compounds are hereby presented and discussed.

The bicyclononanolide structure was derived when augustinolide was compared with a product obtained from mexicanolide[2] (IV) following reduction and acetylation. However, due to structural complications, presented in the sequel, augustidienolide to which structure IIa was assigned will be discussed first.

IIa shows a molecular ion peak, M^+ 510, a UV spectrum λ_{max} 282 mμ (ε 8000) and a set of signals in the NMR spectrum which are given in Table 1. The absorption max at λ 282 mμ indicates an extended conjugated system with a carbonyl group, and the presence in the NMR spectrum of a *single* vinylic proton, in addition to the furanic proton signals, implied that the two double bonds had to be placed in a way which would produce such a single proton, namely at 8–9 and 14–15, the only vinylic proton being at C_{15}. A similar chromophore has been previously observed

TABLE 1. NMR SPECTRA OF ANGUSTIDIENOLIDE (IIa) AND 2α-HYDROXYANGUSTIDIENOLIDE (III) (δ VALUES)

Substance	C_3—H	C_{15}—H	C_{17}—H	α, α′ furan	β furan	CH_3 | CO—O	CH_3—O	CH_3—	C_2—αOH
IIa	4·9 (d) J = 9 c/s	6·24 (s)	5·19 (s)	7·55 (m) 7·45 (m)	6·50 (m)	2·20	3·71	1·20; 1·05; 0·81; 0·79	
III	5·01 (s)	6·33 (s)	5·20 (s)	7·55 (m) 7·45 (m)	6·50 (m)	2·23	3·71	1·28; 1·08; 0·81; 0·75	4·09 (s)

in this series when mexicanolide (IV) was treated with alkali resulting in an irreversible retro-Michael fission observed and extensively described for this compound.[2] However, in that case the UV spectrum of the product is, when taken in neutral ethanolic solution, a combination of two chromophores λ_{max} 264 mμ for the enolized β-diketone in ring A of V and a shoulder at 285 mμ for the diene lactone. Upon addition of alkali, only one max at 287 mμ developed for the enolized β-diketone anion and diene lactone.[2] When alkali was added to an ethanolic solution of IIa no noticeable changes took place in the UV spectrum as should have been expected from a fission similar to that taking place with mexicanolide (IV). Support for structure II was adduced through analysis of the fragmentation pattern of the mass spectrum of the compound in which two characteristic peaks, *m/e* 96 and 414, corresponding to fragments (a) and (b) respectively were identified. In compound IIa a 3β-OAc group is present, observed as a 3-proton signal at δ 2·20 and the 3α-proton geminal to that group appears as a doublet centered at δ 4·91 ($J = 9$ c/s) due to coupling with its neighbouring C_2 α-hydrogen. Hydrolysis with base at room temperature afforded the 3β-OH derivative IIb in which the 3α-proton signal was shifted to δ 4·12, again as a doublet ($J = 7·5$ c/s), while the 3β-OAc group signal had now disappeared. The alcohol group was then oxidized with chromium trioxide in acidic conditions (Jones reagent) affording the corresponding 3-keto derivative IIc, devoid of all the above signals. Compound IIc can thus be considered as a derivative of carapin[3] (VI) having an additional double bond at 8–9.

The second compound to be analysed, 2α-hydroxy-augustidienolide was assigned structure III on the following grounds: its molecular ion peak M^+ 526 had the element of one additional atom of oxygen, while the UV spectrum λ_{max} 282 mμ (ε 7100) is similar to that of IIa, the same is true for most of the signals in the NMR spectrum which are given in Table 1. It could therefore be inferred, from the UV spectrum and the presence of the single vinylic proton signal in the NMR spectrum, that in this compound, the same extended conjugated system is present as in IIa. The location of the additional OH group at C_2 was found by comparing the NMR spectra of both IIa and III. Indeed, in III the 3α-proton appears as a singlet at δ 5·01 since no vicinal proton is present at C_2 for interaction. Furthermore, a signal for the hydroxylic proton of the tertiary OH group appearing as a singlet at δ 4·09 disappeared on exchange with D_2O. Confirmation was obtained from the mass spectrum of III in which a fragment, *m/e* 42, corresponding to the loss of ketene (c) originated from an acetoxy group at C_3 missing an adjacent hydrogen (necessary for elimination as acetic acid), but instead having a tertiary OH group at that place.

During the hydrogenation of IIa over Pd-C, one mole of hydrogen was consumed producing a mixture of three compounds which was resolved by preparative thick layer chromatography. One of the products, which was obtained in about 50% yield, was identical with I, while the mixture of the two others which could not be separated in a pure form showed an NMR spectrum displaying all the signals corresponding to both 3β-acetoxy carapin[3,4a] VII (as compared with the signals of an authentic sample), and 3β-acetoxy mexicanolide[2] (VIII) (discussed later in the sequel). Hydrogenation of IIc over Pt-C allowing the absorption of only one mole of hydrogen yielded a mixture of two compounds observed on a chromatoplate. Separation was performed by preparative thick layer chromatography: the upper spot, 30% was identified as mexicanolide (IV) by comparison with an authentic sample, while the lower spot, 70% of the mixture, M^+ 468, was found to be an isomer of mexicanolide (IV) and its structure IX was determined through its NMR spectrum. The outstanding difference between the spectra of the two isomers was the location of the C_{17}-β proton signal appearing as a singlet at δ 5·30 for mexicanolide (IV) and δ 5·59 for the isomer (IX). This difference would indicate a different location of the double bond in the skeleton. Since no vinylic proton was observed in the NMR spectrum of IX the only place left in the molecule for a tetrasubstituted double bond is at C_{8-9}. Adduced support for this location of the double bond in this compound, for which the name iso-mexicanolide (IX) is proposed, will be given by analysing the positions of the C_{17} β-H signals in the NMR spectra of several compounds of this series given in Table 2.

TABLE 2. LOCATIONS OF C_{17}-H SIGNALS OF VARIOUS TETRANORTRITERPENOIDS (δ UNITS)

Compound	C_{17}—H	Compound	C_{17}—H
Angustinolide I	5.69 (s)	Methyl angolensate[4a]	5·65 (s)
Angustidienolide IIa	5·19 (s)	Swietenine[6]	5·57 (s)
2α-OH Angustidienolide III	5·20 (s)	Carapin VI[3]	5·10 (s)
3β-OH iso-mexicanolide[2] X	5·63 (s)	3β-OH Carapin[3]	5·10 (s)
iso-mexicanolide IX	5·59 (s)	3β-OAc Carapin[3]	5·12 (s)
Mexicanolide IV[2]	5·30 (s)	Enolized β-diketone V[2]	5·10 (s)
Angolensic acid[4a]	5·65 (s)	Andirobin[4]	5·53 (s)

This brings us back to an analysis of the structure of augustinolide (I) (a compound called fissinolide). Fissinolide* was reported to have in its NMR spectrum a singlet at δ 5·69 related to C_{17}-H, a location similar to the signal related to this proton in iso-mexicanolide (IX). When mexicanolide having its C_{17}-H signal at δ 5·30 (s),[2] is reduced with sodium borohydride, a 3β-OH derivative X is obtained with this signal now at δ 5·63. Upon acetylation of X the 3β-OAc derivative (I) is obtained in which the C_{17}-H is at δ 5·69, an identical location with that of augustinolide. From an analysis of the C_{17}-H signals in the compounds discussed in this paper, as well as in several of the tetranortriterpenoids reported by Powell[4a] and by Conolly *et al.*,[2] one can observe two sets of values for this signal. One group ranging from 5·10 to 5·30 as is the case in compounds IV, IIa and III, all having a double bond attached at C_{14} (ether 8–14 or 14–15) and those ranging between 5·55 and 5·70 for compounds X and I (Table 2). Comparing these set of values with those for the C_{17}-H signals reported for angolensic acid and methyl angolensate[4a] δ 5·65, for swietenine[5] δ 5·57 and for andirobin[4] δ 5·53, in all of which no double bond is attached at C_{14}, one can see that these values are very close to those of compounds X and I. Concerning the values falling between 5·10 and 5·30, they can be compared with those observed for carapin[3] (VI), 3β-hydroxycarapin,[4a] 3β-acetoxycarapin[4a] (VII) as well as for compound V,[2] in all of which the double bond is attached at C_{14}.

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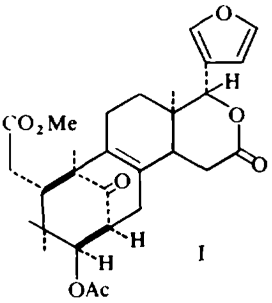
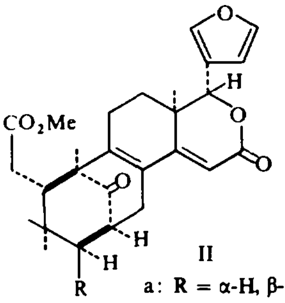
* *Cedrela angustifolia* has been previously classified as *Cedrela fissilis* Vell. (herbarium SP 954 of the botanical garden of São Paulo). The classification of the Meliaceae has been recently revised by Dr. T. C. Rizzini from the botanical garden of Rio de Janeiro. The compound isolated first from *C. fissilis* was given the name fissinolide,[1] but since its structure is hereby revised it was felt that the name angustinolide would fit better its origin, and can be well used as a basis for naming the two other additional compounds.

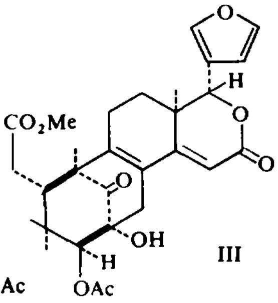


I

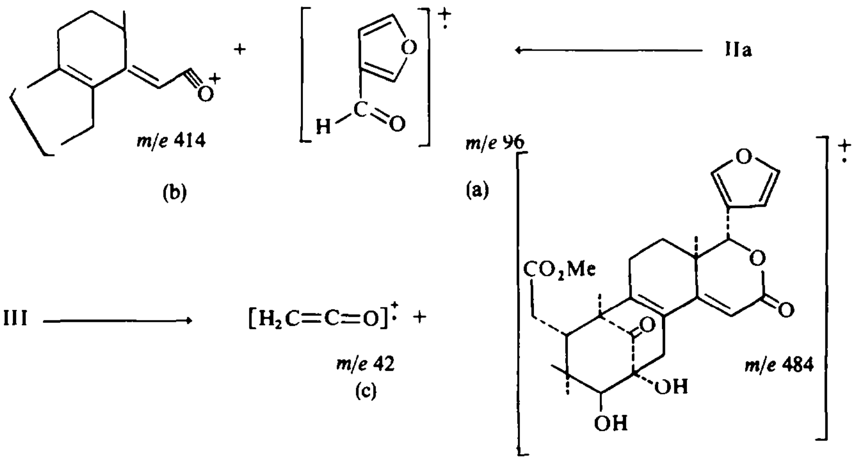


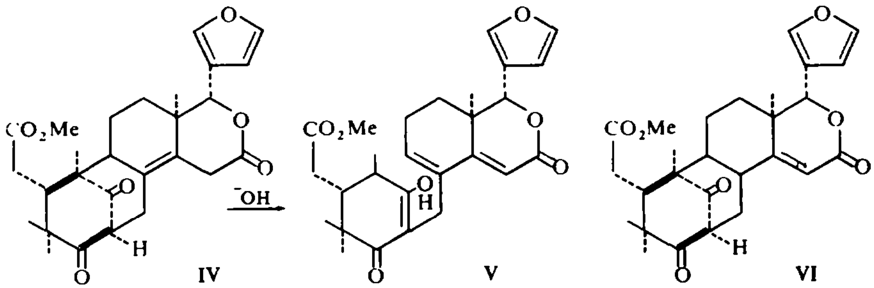
II

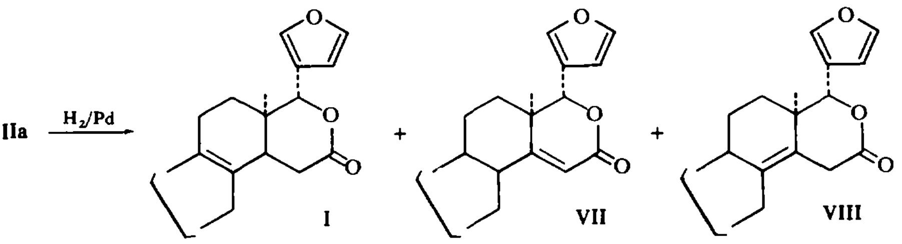
a: R = α-H, β-OAc
b: R = α-H, β-OH
c: R = O

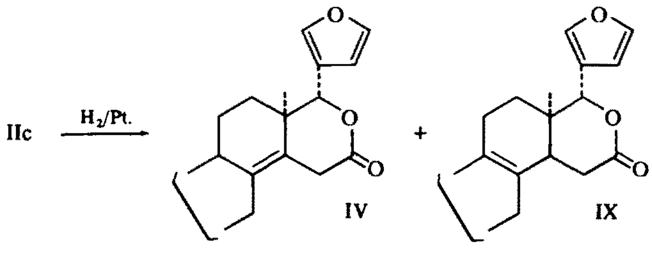


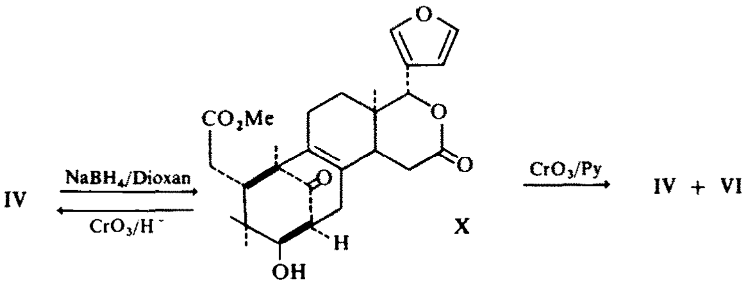
III

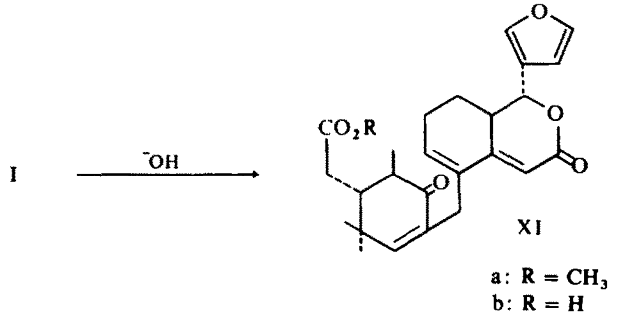












It can be concluded therefore that the 3β-OH derivative X obtained from mexicanolide (IV) and its 3β-OAc derivative I, which is angustinolide should not have their double bonds attached to C_{14} (i.e. at C_{8-9}). Indeed iso-mexicanolide (IX), described above, has this signal at δ 5·59 and therefore in perfect agreement with a double bond not attached at C_{14}. It should thus be concluded that the sodium borohydride reduction product of mexicanolide (IV) yields actually 3β-hydroxy-iso-mexicanolide (X), and its acetylation product presently described as angustinolide (I) *has its double bond at* C_{8-9}.

When X is oxidized with chromium trioxide two different products are obtained according to the conditions of the oxidation. In acidic conditions (acetone solution) mexicanolide (IV) is exclusively obtained in which the double bond has shifted from Δ^8 to $\Delta^{8,(14)}$, while in pyridine a mixture of 60% carapin[3] (VI) (double bond at 14–15) and 40% mexicanolide (IV) were obtained. Both compounds were obtained in a pure form from a preparative chromatoplate, each being identified by comparison with authentic samples.[3,]** These reactions are clear indications of the lability of the double bond between rings C and D.

** The mexicanolide was isolated from seeds of *Cedrela fissilis* Vell. collected in Campinas, São Paulo.

The location of the double bond in the different compounds was further corroborated through their behavior in presence of alkali. While the addition of two drops of 4N NaOH to mexicanolide[2] (IV) as well as to carapin[3] (VI) resulted in the formation of V as indicated in the UV spectrum by an intense absorption at λ_{max} 287 mμ (ε 31,700), iso-mexicanolide (IX) and 3β-hydroxy-iso-mexicanolide (X) did not react and no changes whatsoever were seen in their UV spectra. Interestingly the 3β-OAc derivative I (angustinolide), although having a double bond at C_{8-9} as in IX and X, reacted with alkali to yield the ester XIa at room temperature,[2] or on reflux the corresponding acid[1] (XIb). These cleavage products differed from the one obtained from mexicanolide which had structure (V). The NMR spectrum of XIb conformed with that of the previously described data,[2] however the signal related to the ester Me group was missing.

In the present case elimination of the OAc group takes place first,[2] and the double bond thus formed is clearly seen in the UV spectrum as an inflexion at λ_{max} 236 mμ (ε 12,700) for the unsaturated ketone produced in ring A while the max at 278 mμ (ε 14,600) is due to the diene lactone system.

EXPERIMENTAL

M.ps were taken on a Kofler hot-stage microscope or on a Fischer–Johns apparatus and are uncorrected. The IR spectra were measured in KBr discs on a Unicam SP-200 and a Perkin–Elmer Infracord 137 spectrometer. UV measurements were recorded on a Cary-14 spectrophotometer in EtOH soln. The NMR spectra were recorded on a Varian A-60 instrument, for 5–10% solns in $CDCl_3$ containing TMS as internal standard. Mass spectra measurements were done on an Atlas CH4 spectrometer. Optical rotations were recorded with an automatic Perkin–Elmer polarimeter and refer to $CHCl_3$ solns unless otherwise specified. TLC were carried out on plates coated with silica gel G. Analyses were performed in the microanalytical laboratory of the Weizmann Institute, under the direction of Mr. R. Heller.

Extraction of angustinolide (I), *angustidienolide* (IIa) *and 2α-hydroxyangustidienolide* (III)

The ground seeds (860 g) collected from *Cedrela angustifolia* Sessé and Moc. (botanical garden of the Institute Butantan) were exhaustively extracted with refluxing light petroleum b.p. 35–80° to give upon concentration 77 g of oil. The defatted material was further extracted with refluxing trichlorethylene and concentration of the solvent furnished a viscous mass (99 g) which upon trituration with light petroleum at room temp precipitated as a solid mass (61.3 g). This crude material was chromatographed on a column packed with silica gel H at a daily rate of one elution.

The elutions with benzene–$CHCl_3$ 9:1 and 4:1 gave 130 mg of β-sitosterol, m.p. 132–136°, readily identified by a mixture m.p. and comparison of IR spectra with an authentic sample.

The elution with benzene–$CHCl_3$ 3:1 afforded *angustinolide* (I; 9·9 g) as a crystalline substance from MeOH, which was recrystallized from the same solvent, m.p. 168–171°; $[\alpha]_D$ −185° (*c* 1·9); λ_{max} 213 mμ (ε 10,700); (Found: C, 67·49; H, 7·75; M^+ 512; $C_{29}H_{36}O_8$ requires: C, 67·56; H, 7·85% M wt 512·58).

Elution with benzene–$CHCl_3$ 1:2 afforded a second fraction which crystallized from MeOH to give *angustidienolide* (IIa; 1·78 g), m.p. 248–254°; $[\alpha]_D$ +133° (*c* 2·0); λ_{max} 282 mμ (ε 8000); ν_{max} 1720, 1700, 1590, 1490, 1380, 1250, 1040, 890 cm^{-1} (Found: C, 68·22; H, 6·69; M^+ 510; $C_{29}H_{34}O_8$ requires: C, 68·29; H, 6·71%; M wt 510·56).

Further elution from benzene–$CHCl_3$ 1:2 gave a fraction (120 mg) which crystallized from MeOH to yield *2α-hydroxyangustidienolide* (III; 20 mg); m.p. 210–220°; $[\alpha]_D$ +101° (*c*, 2·0); λ_{max} 282 mμ (ε 7100); ν_{max} 3400, 1720, 1700, 1590, 1510, 1475, 1380, 1240, 1040, 890 cm^{-1}. (Found: M^+ 526; $C_{29}H_{34}O_9$ requires: M. wt. 526·56).

Alkaline hydrolysis of angustinolide (I)

Angustinolide (I; 1·15 g) in 10% methanolic KOH (25 ml) was heated for 10 min under reflux. Concentration of the methanolic soln and dilution with water followed by acidification (H_2SO_4, 2N) afforded a ppt which was filtered off washed free of acid with water and dried. The amorphous white material

(620 mg) was then chromatographed on silicic acid (Merck 0·05–0·20 mm) and the elutions with $CHCl_3$–acetone 4:1 gave a fraction which crystallized from MeOH (150 mg). These were again chromatographed and elution with $CHCl_3$–acetone 4:1 yielded XIb (47 mg) m.p. 210–220°; ν_{max} 1720 (COOH), 1670 (unsaturated ketone), 1500–870 (furan) cm^{-1}; λ_{max} 215 (furan), 235 infl. (unsaturated ketone), 278 mμ (trans-dienoic lactone). NMR (δ): 6·25 s (H_3), 6·08 m (H_9), 5·78 s (H_{15}), 5·13 s (H_{17}), 7·46 m ($H_{21,23}$), 6·43 m (H_{22}), 1·03–1·15 (4 × CH_3).

Sodium borohydride reduction of mexicanolide[**] (IV)

(a) 3β-*Hydroxy-iso-mexicanolide* (X). A mixture of IV (1·39 g), $NaBH_4$ (1·4 g) and MeOH (500 ml) were stirred for 20 min in the cold, then worked up in the usual way. The ppt was washed with water and dried to give a crude product (980 mg) which was chromatographed on silicic acid. Elutions with ether gave X (66 mg), crystals from ether, m.p. 182–186°. Evaporation of the mother-liquour left an amorphous residue (480 mg) which was rechromatographed. Elution with ether–benzene (1:3) gave after crystallization from ether a second crop of X (100 mg). Several recrystallizations of the combined crops from ether yielded pure X (60 mg), m.p. 188–192°; $[\alpha]_D$ −141° (*c* 1·5); ν_{max} 3400 (OH), 1730 (δ-lactone), 1700 (C═O), 1510–890 (furan), 1040 (C—O—C) cm^{-1}.

In a second experiment IV (135 mg) was dissolved in dioxan (30 ml), $NaBH_4$ (130 mg) was added and the mixture stirred at room temp for 15 min. The product was then poured onto water, acidified to pH 7 and extracted with $CHCl_3$. The residue (100 mg) was applied on thick layer chromatoplates irrigated with $CHCl_3$–MeOH (98–2), and 4 distinct spots were observed. The major uppermost spot (30 mg) crystallized from ether and proved to be X.

(b) 3β-*Acetoxy-isomexicanolide* (I). A mixture of IV (2 g), $NaBH_4$ (1 g) and MeOH (500 ml) was stirred for 90 min at room temp then worked up in the usual way. The ppt (1·84 g) was acetylated (Ac_2O–pyridine) and the concentrate (950 mg) was chromatographed on silicic acid. Elutions with benzene gave the 3β-OAc compound (502 mg) which crystallized from MeOH, m.p. 168–172°. This compound was found identical with I, underpressed mixture m.p. and IR spectra superimposable throughout the whole range.

Oxidation of 3β-*hydroxy-iso-mexicanolide* (X)

(a) *Chromium trioxide in acidic conditions.* Compound X (20 mg) was dissolved in acetone (20 ml), and to the ice cooled soln (5°) three drops of Jones reagent were added and the mixture stirred for 5 min. After work up the $CHCl_3$ extract was dried over Na_2SO_4 and the solvent evaporated. The product (15 mg) crystallized from a mixture of $CHCl_3$–MeOH to yield exclusively IV, undepressed mixture m.p., the IR as well as the NMR spectra of the product were identical with that of an authentic sample.[**]

(b) *Chromium trioxide in pyridine.* To X (60 mg) in dry pyridine (10 ml) a soln of CrO_3 (100 mg) in dry pyridine (10 ml) was added dropwise. The resulting mixture was stirred at room temp for 3 hr, poured onto water and extracted with $CHCl_3$, this soln was dried over Na_2SO_4 and then passed through a small silica-H column and eluted with $CHCl_3$. The residue left after evaporation of the solvent showed two spots on a chromatoplate irrigated with EtOAc light petroleum (3:2). Separation was done on thick layer chromatoplates irrigated with the same solvent mixture. The upper spot proved to be IV (25 mg) by comparison with an authentic sample. The lower spot was identified as VI (35 mg), m.p. and undepressed mixed m.p., 176–178°, IR and NMR spectra identical with that of an authentic sample.

Catalytic hydrogenation of angustidienolide (IIa)

Angustidienolide (IIa; 100 mg) in EtOAc soln (15 ml) was hydrogenated over Pd–C (30 mg) at atm press. One mole of H_2 (4·4 ml) was absorbed within 15 min. The soln was collected and passed through a silica-H plug eluted with $CHCl_3$. The solvent was removed *in vacuo* and the residue showed two spots on a chromatoplate irrigated with EtOAc–light petroleum (1:1. Separation was performed on thick layer chromatoplates with the same solvent mixture. The upper spot (50 mg) was identified as pure I by comparison with an authentic sample.[1,*] The lower spot could not be induced to crystallize and was shown by NMR spectroscopy to consist of a mixture of VII and VIII.

Alkaline hydrolysis of angustidienolide (IIa)

To a soln of IIa (300 mg) in MeOH (100 ml), a 10% soln of methanolic NaOH (0·3 ml) was added dropwise. The soln was then left overnight at room temp. Acid was added to pH 7 and the soln diluted with ice-water. The product was extracted with $CHCl_3$ the soln dried over Na_2SO_4 and evaporated. The product, one major spot on a chromatoplate irrigated with $CHCl_3$–MeOH (9:1), was purified on a thick layer chromatoplate to yield pure IIb (250 mg), crystallized from MeOH m.p. 194–196°; $[\alpha]_D$ +271° (*c* 2·0); λ_{max} 282 mμ (ε 7400), (Found: M^+ 468; $C_{27}H_{32}O_7$ requires: M wt. 468·55).

Oxidation of 3β-*hydroxyangustidienolide* (IIb) *to* 3-*ketoangustidienolide* (IIc)

The above IIb (240 mg) in acetone soln (50 ml) was oxidated with Jones reagent (3 ml) at room temp for 5 min. After the usual work up the $CHCl_3$ extract was dried over Na_2SO_4 and passed through a silica-H plug. The solvent was removed and the product purified on a thick layer chromatoplate irrigated with $CHCl_3$–MeOH (98:2); 3-keto-angustidienolide (150 mg) crystallized from MeOH, m.p. 101–103°; $[\alpha]_D$ +84° (*c* 2·0); λ_{max} 283 mμ (ε 9000); (Found: M^+ 466; $C_{27}H_{30}O_7$ requires: M. wt. 466·53).

Catalytic hydrogenation of 3-*ketoangustidienolide* (IIc)

Compound IIc (100 mg) in EtOAc soln (40 ml) was hydrogenated over Pt catalyst (30 mg) at atm press. After absorption of one mole H_2 the soln was filtered through a small column of silica gel and eluted with $CHCl_3$. The crude product (90 mg) showed two spots on a chromatoplate irrigated with EtOAc–light petroleum (3:2). Separation was done on a thick layer chromatoplate using the same solvent mixture: the upper spot was IV (20 mg), undepressed mixture, m.p. 222–226°, and IR and NMR spectra identical with an authentic sample, the lower spot *isomexicanolide* (IX 60 mg) crystallized from a $CHCl_3$–MeOH; m.p. 234–236°, $[\alpha]_D$ −24° (*c*, 2·0), λ_{max} 210 (ε 11,000); (Found: C, 69·15; H, 6·79; M^+ 468; $C_{27}H_{32}O_7$ requires: C, 69·21; H, 6·89%; M wt 468·53).

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REFERENCES

[1] R. Zelnik and C. M. Rosito, *Tetrahedron Letters* 6441 (1966).
[2] J. D. Conolly, R. McCrindle and K. H. Overton, *Tetrahedron* **24**, 1489 (1968).
[3] E. O. Arene, C. W. L. Bevan, J. W. Powell and D. A. H. Taylor, *Chem. Comm.* 302 (1965).
[4] [a] J. W. Powell, *J. Chem. Soc.* (C), 1794 (1966);
[b] W. D. Ollis, A. D. Ward and R. Zelnik, *Tetrahedron Letters* 2607 (1964).
[5] J. D. Connolly, R. Henderson, R. McCrindle, K. H. Overton and N. S. Bhacca, *J. Chem. Soc.* 1281, 6935 (1965).