Constituents of Commercial Tolu Balsam

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The hexane-soluble fraction of an ethanolic solution of commercial tolu balsam, accounting for most of the aroma, is shown to be a complex mixture, composed of aromatic and terpenoid compounds. Benzoic and cinnamic acids, the corresponding alcohols, aldehydes, ethyl, benzyl, and cinnamyl esters, methyl cinnamate, styrene, eugenol, vanillin, benzyl ferulate or isoferulate, ferulic acid, and 1,2-diphenylethane are the aromatic compounds identified. A number of mono- and sesquiterpene hydrocarbons and alcohols are present in low concentration, of which three mono- and fourteen sesquiterpene hydrocarbons were identified (Table 1). The balsam also contains numerous triterpenoids including dammaradienone, 20R-hydroxy-dammarenone, 20S-dammarenediol, hydroxyhopanone, oleanolic acid, sumaresinolic acid, 3-oxo-6β-hydroxyolean-12-en-28-oic acid, urs-12-en-3-on-28-al, and 20R,24ξ₂-ocotillone, of which the last three are new.

Tolu balsam is a commercially available bled resin from a tree, *Myroxylon balsamum* (L.) Harms (Leguminosae), endemic to the northern part of South America. The balsam has a sweet, pleasant and lasting aroma and is also considered to exhibit a certain fixative effect. It is commonly used as an ingredient in confectionary products and cosmetics and as an additive to tobacco.

Early investigations on the chemical composition, summarised by Rollett,[1] have shown that benzoic and cinnamic acids, the corresponding benzyl esters and trace amounts of vanillin and farnesol are present in tolu balsam. Moreover, eugenol, coniferyl alcohol, and coniferyl cinnamate have been indicated by the same author as probable constituents.[1] Later, Naves[2] has confirmed the presence of benzyl benzoate and farnesol in tolu balsam and has also demonstrated that the essential oil from the wood of *M. balsamum* (L.) Harms contains *l*-cadinol, *d*-cadinene, farnesol and nerolidol. One further compound, 3',4',7-trimethoxyisoflavone (cabreuvin) has been isolated from the wood; this compound has also been encountered in the wood of the closely related *Myrocarpus fastigiatus* Fr. Allem (Leguminosae).[3] On dry distillation, tolu balsam has been shown to give benzoic and cinnamic acids, guaiacol, homoguaiacol, and probably ethylguaiacol.[4]

The available knowledge on the chemical composition of this complex material is thus somewhat sparse and the present investigation [5,6] was undertaken with a view to identifying further significant flavour constituents as well as some less polar major components. The examination was limited to the hexane-soluble part of an ethanolic solution of tolu balsam, as this accounts for most of the aroma.

RESULTS

The hexane-soluble part (32 %) of an ethanolic solution of commercial tolu balsam [7] was separated into fractions containing strong acids (12 %), weak acids (2.4 %), and neutrals (17 %).

Neutrals. The neutral fraction was the most important one from a flavour point of view. According to TLC it was also the most complex one, composed of compounds of widely different polarity. It was therefore initially separated into a hydrocarbon fraction A (1.6 %), a minor fraction B (0.06 %), which proved to consist of polar hydrocarbons, and a major fraction C (15.4 %), comprising oxygenated compounds.

Fraction A, having a faint celery-like aroma, was highly complex, and was hence separated further by distillation, using a recently described technique.[8] The volatile material was subsequently divided by liquid chromatography into saturated and unsaturated hydrocarbons. The latter constituents, comprising all of the flavour compounds, were investigated further, while the saturated hydrocarbons were discarded. According to GLC in combination with mass spectrometry the unsaturated hydrocarbon fraction was mainly composed of sesquiterpenes with monoterpenes and styrene as minor components (Fig. 1). Although some constituents could be recognized solely by their mass spectra, it was considered desirable in this particular case to isolate individual compounds in order to confirm the mass spectrometric results and to permit the identification of further constituents. The unsaturated hydrocarbons were therefore chromatographed on $AgNO_3$-impregnated silica gel, and the main components of the fractions obtained were isolated by preparative GLC to give the compounds presented in Table 1.[9] The presence of α-curcumene,

Table 1. Unsaturated hydrocarbons in tolu balsam. (Peak numbers refer to Fig. 1.)

Peak No.	Compound	Peak No.	Compound
1	α-Pinene	11	α-Curcumene
2	Styrene	12	γ-Muurolene
3	cis-β-Ocimene	13	Unidentified
4	p-Cymene	14	α-Muurolene
5	Unidentified	15	β-Selinene
6	α-Bourbonene	16	δ-Cadinene
7	α-Copaene	17	γ-Cadinene
8	β-Bourbonene	18	Calamenene
9	β-Elemene	19	α-Calacorene
10	Caryophyllene		

however, was only established by mass spectrometry, since it was a very minor component and could not be isolated in sufficient amount for further spectroscopic studies. Two of the hydrocarbons (5 and 13), probably sesquiterpenes, may be new compounds, since they gave spectra which do not correspond with available reference spectra.
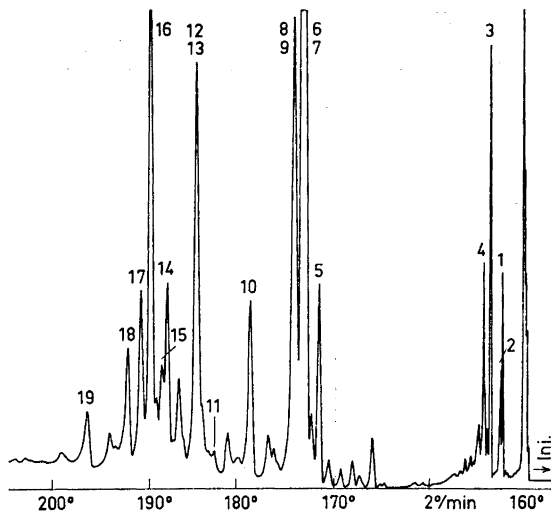

Fig. 1. Gas chromatogram of tolu balsam: unsaturated hydrocarbons.

Fraction *B* yielded cadalene and 1,2-diphenylethane. To our knowledge the latter hydrocarbon has previously not been encountered in plant material. However, natural products having the same carbon skeleton are known, *e.g.* pinosylvin,[10] and these two compounds may well be formed *via* a similar biosynthetic route.

The oxygenated neutral material, fraction *C*, proved to be very complex and was shown to contain compounds of greatly differing volatilities. It was therefore distilled to give a volatile fraction (5.7 %), accounting for the heavy and sweet part of the aroma of tolu balsam, and a residue (9.7 %) having a less characteristic and pronounced aroma. The gas chromatogram (Fig. 2) showed that the distillate was composed of relatively few major constituents with several minor ones. A gas chromatographic-mass spectrometric examination of the distillate and of fractions obtained from it by liquid chromatography revealed that the main peaks were due to a series of aromatic compounds, while the minor peaks mainly corresponded to mono- and sesquiterpene alcohols. The previously known tolu component, benzyl benzoate, was the predominant constituent (Table 2). Other significant components were benzaldehyde, cinnamaldehyde, benzyl alcohol, cinnamyl alcohol, methyl cinnamate, benzyl cinnamate, cinnamyl benzoate, ethyl benzoate, and ethyl cinnamate. It must be noted, however, that the ethyl esters may have been

formed during the storage of the tolu balsam in ethanol. The presence of all of these compounds was established solely by means of GLC and mass spectrometry as the mass spectra are sufficiently characteristic to permit unambiguous identification. However, the presence of both benzyl cinnamate and cinnamyl benzoate, indistinguishable by GLC on the Apiezon L column, was confirmed by GLC-MS carried out on a packed OV-17 column. Many of these aromatic constituents, and probably several of the terpenoid alcohols, make important contributions to the aroma of tolu balsam. The terpenoid alcohols were not isolated and identified but they would be expected to possess carbon skeletons similar to those of the hydrocarbons identified.
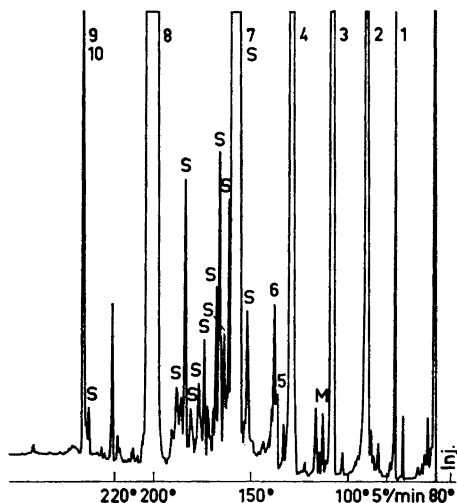

Fig. 2. Gas chromatogram of tolu balsam: neutral oxygenated compounds.

With the exception of cinnamyl cinnamate and some further benzyl cinnamate and cinnamyl benzoate, the non-volatile part of fraction C was composed of triterpenoids. The separation of these compounds proved difficult on account of the large number of constituents present and the limited number available in isolable quantity. The occurrence of both tetracyclic and pentacyclic constituents could be established, and those incorporating a dammarane skeleton

Table 2. Oxygenated compounds in tolu balsam. (Peak numbers refer to Fig. 2.)

Peak No.	Compound	Peak No.	Compound
1	Benzaldehyde	7	Ethyl cinnamate
2	Benzyl alcohol	8	Benzyl benzoate
3	Ethyl benzoate	9	Benzyl cinnamate
4	Cinnamaldehyde	10	Cinnamyl benzoate
5	Cinnamic alcohol	M	Monoterpene alcohol
6	Methyl cinnamate	S	Sesquiterpene alcohol

were shown to be predominant. Thus, dammaradienone (*1*), 20*R*-hydroxy-dammarenone (*2*) and 20*S*-dammarendiol (*3*) were obtained in a pure state, while the presence of the 20-epimers of the last two compounds could not be established with certainty. Another compound of dammarane type, having an ether-bridged side-chain, was isolated and found to be a hitherto unknown ocotillone isomer, designated as 20*R*,24ξ_2-ocotillone (*4*). The structure determination of this compound has been described elsewhere.[6] Moreover, urs-12-en-3-on-28-al (*5*), apparently a new naturally occurring pentacyclic triterpenoid, was found to be present. It was identified by conversion to the corresponding diol, uvaol. The mass spectrometric results indicated the presence of further derivatives of α- or β-amyrin type but none of these were obtained in a pure state. One hopane derivative, hydroxyhopanone (*6*), was isolated and identified.

1

2

3

4

5

6
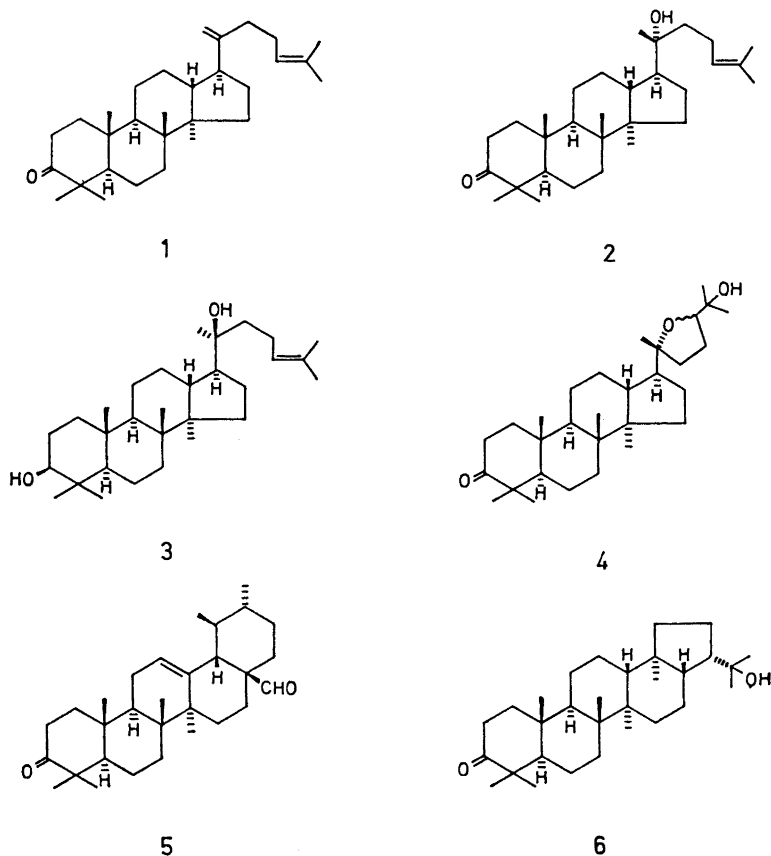
Weak acids. The weak acids constituted a rather complex mixture, and isolation of the components was accomplished mainly by repeated liquid chromatography and recrystallization. The reported presence of eugenol and van-

illin in tolu balsam, both isolable from two of the least polar fractions, was confirmed. Another phenolic constituent could only be isolated after methylation and subsequent acetylation of a more polar fraction. The compound was found to be a substituted cinnamyl ester which on saponification gave benzyl alcohol and 3,4-dimethoxycinnamic acid, demonstrating that the corresponding tolu component was benzyl ferulate or isoferulate. The major part of the weak acids was, however, found to consist of triterpene acids, of which there were three main components. These were separated and converted, using diazomethane, to the corresponding methyl esters which were subsequently purified further. The identification of two of these methyl esters as methyl oleanolate (7) and methyl sumaresinolate (8), and the structure determination of the third as methyl 3-oxo-6β-hydroxyolean-12-en-28-oate (9) have been described in an earlier publication.[5]

7   R_1 = H,OH(β) R_2 = H
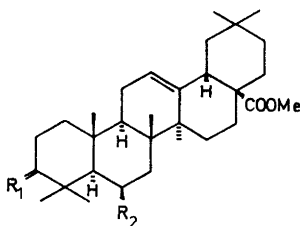
8   R_1 = H,OH(β) R_2 = OH

9   R_1 = O R_2 = OH

Strong acids. In sharp contrast to the complexity shown by the fractions described hitherto, the only abundant strong acids present in the hexane-soluble part of tolu balsam were found to be cinnamic acid and benzoic acid. Cinnamic acid, an important flavour constituent, was the predominant compound. Furthermore, traces of an acid, showing chromatographic and mass spectral properties identical to those of ferulic acid, were encountered.

EXPERIMENTAL

M.p.'s were determined on a Kofler micro hot-stage and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 257 instrument and NMR spectra on a Varian A-60A spectrometer at 60 MHz. Rotations were measured on a Perkin-Elmer 141 polarimeter.

Preparative GLC was carried out on a Varian 1700 instrument, equipped with a flame ionisation detector and an outlet splitter (ratio 1 : 50). The temperature of the injection block and the detector was kept at 275°. A 2 m glass column (i.d. 9.6 mm) packed with 10 % Carbowax 20 M on Chromosorb G support, and a 2 m steel column (i.d. 9.6 mm) packed with 3 % OV 17 on the same support were used. The carrier gas flow was 150 ml N_2/min and the effluents from the column were collected in teflon tubing cooled with dry ice. Aerosols were precipitated utilizing a modification of an electrostatic method based on the Cottrell principle.[11]

Analytical GLC was performed on the same type of instrument but equipped with a capillary injector using either a 50 m steel column (i.d. 0.5 mm) coated with SF 96, a 30 m steel column (i.d. 0.5 mm) coated with Apiezon L or a 2 m steel column (i.d. 3.2 mm) packed with 3.5 % OV-17 on Chromosorb W support. The carrier gas flow was 3 ml N_2/min (capillary column) or 30 ml N_2/min (packed column) and the samples were injected on the capillary columns with a split ratio of 1 : 20. GLC in combination with mass spectrometry was carried out on an LKB 9000 instrument, incorporating a home-made gas chromatograph equipped with both a capillary and a packed column injector, a device for introduction of make-up gas, a splitter and a flame ionisation detector. The columns described were used and nitrogen was substituted for helium as a carrier gas. The temperature of the separator was 260° and that of the ion source 290°. The electron energy used was 70 eV. Mass spectra of non-volatile compounds were recorded using the direct inlet system.

Liquid chromatography was carried out on silica gel (Merck 0.05 − 0.20 mm, activity I), on neutral alumina (Schuchardt, activity II), and on $AgNO_3$-impregnated silica gel.[12] Thin-layer chromatography was performed on silica gel Merck G or GF and on silica gel impregnated with $AgNO_3$.[12]

Fractionation. The tolu balsam used was a commercial material, obtained from the Pomade of Peru Co., London, and originating from Colombia. The properties of this material were in accordance with the requirements of the Pharmacopeia of Sweden, 1946. In view of the complexity of the balsam, several batches of material were processed with slight modifications of the procedures. The fractionation of one batch is described in the paragraph below, while the description of the further separation of the neutral fraction refers to a later batch.

Tolu balsam (819 g), dissolved in a volume of 1 l of ethanol, was extracted twice with hexane (2 × 5 l). The combined hexane extracts were carefully concentrated, to give a crystalline material (266 g, 32 %). This was recrystallized from cyclohexane and cyclohexane − isopropyl ether yielding cinnamic acid (38 g, 4.6 %), m.p. and mixed m.p. 134 − 136°; the IR and mass spectrum and the R_F-value on TLC were identical to those of authentic material. After careful evaporation of solvent, the residue was dissolved in light petroleum (35 − 60°) and extracted five times with a saturated aqueous $NaHCO_3$ solution. On acidification of the combined aqueous solutions with dilute H_2SO_4 (10 %), a precipitate was formed. This was filtered off and shown to consist of cinnamic acid (46 g, 5.6 %). The aqueous solution was then extracted with ether. The ether phase was washed with water, dried with Na_2SO_4 and concentrated to give a fraction comprising the remaining strong acids (12 g, 1.5 %). The light petroleum phase remaining after $NaHCO_3^-$ treatment was extracted five times with aqueous NaOH (10 %). The alkaline phase thus obtained was acidified with H_2SO_4 (10 %) and extracted with ether to give the weak acids (20 g, 2.4 %). The light petroleum solution was then washed with water, dried and concentrated to give the neutrals (142 g, 17 %).

Neutrals. The neutral material (241 g*) was separated into a main hydrocarbon fraction *A* (23 g, 1.6 %) a fraction comprising polar hydrocarbons *B* (0.8 g, 0.06 %), and a fraction containing oxygenated compounds *C* (217 g, 15.5 %) by chromatography on neutral alumina (pentane − ether − ethanol).

The main hydrocarbon fraction *A* (23 g) was distilled at reduced pressure (90°, 0.01 − 0.001 mmHg) using CO_2 as a carrier gas [8] to give a distillate (18 g, 1.3 %) and a distillation residue (4.6 g, 0.3 %), which was discarded. The distillate was chromatographed on $AgNO_3$-impregnated silica gel (pentane − ether) to give eleven fractions. These were examined by GLC-MS (SF 96). Fraction 1 (5.5 g) consisted of saturated hydrocarbons, while fractions 2 − 11 contained unsaturated hydrocarbons and were subjected to preparative GLC. Spectral data were recorded on isolated components having a purity of 95 % or better according to GLC on capillary columns. Table 3 lists the isolated and identified components and shows which reference spectra were used for their identification.

Two unidentified sesquiterpene hydrocarbons were isolated from fractions 5 and 3. The former compound, corresponding to peak 5, provided the following spectral data: $[\alpha]_D − 28°$ (CHCl$_3$, c 1.1); ν_{max}(film): 3075, 1665, 1462, 1450, 1405, 1380, 1372, 1364,

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* Since some solvent was left in fractions containing volatile material, the weights given for these are necessarily inexact.

Table 3. Unsaturated hydrocarbons in fraction A.

Fraction No.	Weight, g	Components identified after preparative GLC	Identification based on comparison of		
			IR	NMR	MS
2	1.9	α-Pinene	X[13]	X[13]	X[13]
		p-Cymene	X[13]	X[13]	X[13]
		α-Bourbonene	X[14]		
		Calamenene	X[15]	X[16,17]	X[18]
3	1.1	α-Pinene	X[13]	X[13]	X[13]
		α-Copaene	X[15]	X[16]	X[18]
		Calamenene	X[15]	X[16,17]	X[18]
		α-Calacorene	X[19]		
4	0.2	δ-Cadinene	X[15]		X[18]
		α-Calacorene	X[19]		
5	1.9	cis-β-Ocimene	X[20]	X[20]	X[20]
		Styrene	X[13]	X[13]	X[13]
		β-Bourbonene	X[15]		
		α-Muurolene	X[21]	X[21]	X[18]
		δ-Cadinene	X[15]		X[18]
6	0.8	cis-β-Ocimene	X[20]	X[20]	X[20]
		γ-Muurolene	X[21]	X[16,21]	X[18]
		γ-Cadinene	X[13,15]	X[13]	X[13,18]
		α-Curcumene [a]			X[18]
7	0.2	γ-Cadinene	X[13,15]	X[13]	X[13,18]
8	1.0				
9	0.1	Caryophyllene	X[15]		X[18]
		β-Selinene	X[13,15]	X[13]	X[13,18]
10	1.3	Caryophyllene	X[15]		X[18]
11	1.5	β-Elemene	X[15]		X[18]

[a] This compound was not isolated.

883, 812 cm^{-1}; δ(CDCl$_3$): 0.98, 1.02, 1.07 (all 3H, s), 4.61 (1H, d, $J=1.5$), 4.66 (1H, d, $J=1.5$); m/e (%): 204 (20), 189 (28), 148 (19), 147 (41), 133 (16), 119 (16), 109 (18), 108 (30), 107 (29), 105 (22), 95 (100), 94 (66), 93 (34), 91 (38), 79 (42), 77 (24), 55 (24), 41 (36). The latter sesquiterpene, corresponding to peak 13, showed $[\alpha]_D$ $-27°$(CHCl$_3$, c 0.7); ν_{max}(film): 1675, 1450, 1434, 1370, 807 cm^{-1}; δ(CDCl$_3$): 0.95 (broad signal), 1.00 (sharp signal), 5.30 (1H, broad); m/e (%): 204 (25), 189 (100), 163 (18), 148 (9), 147 (11), 134 (12), 133 (25), 119 (12), 107 (14), 105 (16), 93 (11), 91 (20), 81 (12), 79 (10), 55 (10), 41 (12).

Fraction B (0.8 g) was chromatographed on silica gel (light petroleum-isopropyl ether) to give seven fractions. It was demonstrated by GLC-MS that styrene and α-calacorene, also present in fraction A, were constituents of fraction 3. Cadalene (IR[19]) and 1.2-diphenylethane (IR,[22] MS[23]) were isolated from fractions 3 and 5, resp., by preparative GLC.

Fraction C (217 g), containing oxygenated neutral constituents, was distilled in the same way as fraction A (95°, 0.01 – 0.001 mmHg).[8] The distillate obtained (80 g, 5.7 %) was studied by GLC-MS (Apiezon L). Part of the distillate (5 g) was then subjected to chromatography on neutral alumina (hexane-methylene chloride-ether) to give several relatively simple fractions, which were submitted to studies by GLC-MS (Apiezon L, OV-17).

Part of the residue from the distillation of fraction C (11 g) was chromatographed on neutral alumina (hexane-methylene chloride-ethanol), to give fractions 1 – 8.

Mass spectrometry indicated that fraction 2 constituted a mixture of benzyl cinnamate, cinnamyl benzoate, cinnamyl cinnamate, and triterpenoids. The fraction (2.28 g) was refluxed with aqueous KOH (45 %, 8 ml) in ethanol (25 ml) for 45 min under N_2. Dilution with water and extraction with ether gave a neutral material (1.48 g). The aqueous phase was then acidified with dilute H_2SO_4 (10 %) and extracted with ether to give an acidic fraction (0.8 g), which according to mass spectrometry was a mixture of benzoic and cinnamic acids. The neutral material (1.48 g) on chromatography on silica gel (light petroleum-isopropyl ether-ether) yielded benzyl alcohol, cinnamyl alcohol, and a mixture of triterpenoids (0.6 g). Subsequent chromatography of the triterpenoids on $AgNO_3$-impregnated silica gel (light petroleum-isopropyl ether-ether) gave dammaradienone (1), m.p. and mixed m.p. $77-79°$; $[\alpha]_D + 86°$ (CHCl$_3$, c 1.0) (reported $+90°$).[24,25] The IR, NMR, and mass spectra were identical to authentic spectra; ν_{max} (CCl$_4$): 3070, 1708, 1638, 890 cm^{-1}; δ(CDCl$_3$): 0.90, 0.97, 1.04, 1.06, 1.10, 1.63, 1.70 (all 3H, s), 4.75 (2H, s), 5.15 (1H, t); m/e: 424 (100), 409 (7), 381 (15), 315 (13), 313 (14), 245 (11), 218 (4), 205 (60), 189 (28), 109 (67).

Fraction 4 (0.8 g) consisted, according to mass spectrometry, mainly of an oxoaldehyde of α- or β-amyrin type, ν_{max} (film): 1725, 1705 cm^{-1}; m/e 438, 409, 232, 205, 203, 189, but it could not be obtained in a pure state by chromatography or recrystallization. The fraction was therefore refluxed with LiAlH$_4$ in ether (30 ml) for 30 min. Excess reagent was destroyed with ethyl acetate, and the reaction mixture was diluted with aqueous H_2SO_4 (20 %). Extraction with ether followed by repeated column chromatography on silica gel (light petroleum – isopropyl ether; hexane-acetone) and recrystallizations yielded a crystalline material which was homogeneous on TLC. The IR and mass spectra were identical to those of uvaol. The NMR spectrum revealed, however, that in addition to uvaol, small amounts of another compound, probably erythrodiol, were present. M.p. $215-218°$; mixed m.p. $217-222°$, $[\alpha]_D + 68$ (CHCl$_3$, c 0.5); ν_{max} (KBr): 3370 cm^{-1}; δ(CDCl$_3$): 0.80, 0.95, 1.02, 1.12, corresponding to seven methyl groups, 3.24 (1H, d, $J=10.5$), 3.54 (1H, d, $J=10.5$), 3.70 (1H, t), 5.20 (1H, m); m/e (%): 442 (1), 424 (2), 406 (3), 234 (12), 207 (10), 203 (100), 189 (11), 133 (25).

Fraction 6 (900 mg) was rechromatographed on neutral alumina (light petroleum – isopropyl ether), to give a main fraction (720 mg), which on recrystallization from isopropyl ether – acetone and then from isopropyl ether yielded as a minor component hydroxyhopanone (6), m.p. $250-253°$, mixed m.p. $249-252°$; $[\alpha]_D + 65°$ (CHCl$_3$, c 1.0); reported $+69°$;[24,26] the IR, NMR, and mass spectra were identical to those of authentic material; ν_{max} (KBr): 3470, 1708, 1685 cm^{-1}; δ(CDCl$_3$: 0.77, 0.93, 0.96, 1.00, 1.01, 1.08, 1.17, 1.21 (all 3H, s); m/e (%): 442 (4), 427 (3), 424 (12), 409 (9), 384 (16), 381 (10), 207 (29), 205 (35), 189 (88), 149 (100), 95 (72), 59 (86). The mother-liquors, on subsequent recrystallization from ethanol, gave the major component 20R-hydroxydammarenone (2), m.p. $144.5-146°$; mixed m.p. $143-145°$; $[\alpha]_D + 67°$ (CHCl$_3$, c 0.5); reported $+60°$;[24,27] the IR, NMR, and mass spectra were identical to those of authentic material; ν_{max} (KBr): 3500, 1692 cm^{-1}; δ(CDCl$_3$): 0.90, 0.96, 1.02, 1.05, 1.09, 1.14, 1.64, 1.69 (all 3H, s), 5.14 (1H, t); m/e (%): 424 (100), 409 (7), 381 (10), 355 (28), 315 (13), 313 (21), 205 (48), 189 (17), 109 (72).

Fraction 7 (1.1 g) was chromatographed on silica gel (hexane – methylene chloride) and on $AgNO_3$-impregnated silica gel (light petroleum – chloroform). The main fraction (590 mg) obtained was recrystallised several times from acetonitrile and isopropyl ether to give small amounts of 20R,24ξ_2-ocotillone (4), m.p. $204-206°$; $[\alpha]_D + 54°$ (CHCl$_3$, c 1.0); $M - 15 = 443.3518$; $C_{29}H_{47}O_3$ requires 443.3525; $M - 18 = 440.3650$, $C_{30}H_{48}O_2$ requires 440.3654. The spectroscopic data for this new ocotillone isomer have been given elsewhere.[6]

Fraction 8 (620 mg) on chromatography on silica gel (hexane – acetone) and recrystallization from methanol and nitromethane gave 20S-dammarendiol (3), m.p. $130.5-133°$; mixed m.p. $129-132°$; $[\alpha]_D + 30°$ (CHCl$_3$, c 0.9) (reported $+33°$),[24,27] the IR, NMR, and mass spectra were identical to those of authentic material. ν_{max} (KBr): 3600, 3450, 3410 cm^{-1}; δ(CDCl$_3$): 0.78, 0.87, 0.89, 1.15, 1.64, 1.70 (all 3H, s), 0.98 (6H, s), 3.21 (1H, t), 5.15 (1H, t); m/e (%): 426 (56), 411 (6), 408 (2), 393 (2), 383 (4), 365 (2), 357 (25), 315 (9), 313 (6), 207 (48), 191 (28), 189 (30), 135 (46), 109 (100).

Weak acids. The weak acids (20 g) were chromatographed on silica gel (hexane – acetone) to give fractions $1-8$, partly overlapping and of varying homogeneity. Preparative TLC (light petroleum – isopropyl ether 1:4) of fraction 2 gave eugenol, identified by comparison

of the IR and mass spectra with those of authentic material. A small amount of vanillin, m.p. and mixed m.p. 79.5 – 80°, was obtained by repeated recrystallizations of fraction 3. Fractions 5, 7, and 8 (1.5 g, 3.6 g and 3.2 g) were quite homogeneous and contained triterpene acids according to the mass spectral results. They were treated with ethereal diazomethane for 24 h and subsequently purified by chromatography on silica gel (hexane – acetone) to give the corresponding methyl esters. The least polar of these, on recrystallization from acetonitrile, gave methyl oleanolate (7), m.p. 199 – 201°, mixed m.p. 198 – 201°; $[\alpha]_D + 72°$ (CHCl$_3$, c 0.98); the IR, NMR, and mass spectra were identical to those of authentic material. The second most polar methyl ester was a compound not previously identified as a natural product. The structure determination of this component, methyl 3-oxo-6β-hydroxyolean-12-en-28-oate (9), has been described elsewhere.[5] It was obtained in a pure state by recrystallization from a mixture of hexane and isopropyl ether, and had m.p. 190 – 191°, $[\alpha]_D + 33°$ (CHCl$_3$, c 0.98); (Found: C 76.95; H 10.00. C$_{31}$H$_{48}$O$_4$ requires C 76.82; H 9.98.) The most polar methyl ester, on recrystallization from acetonitrile, gave methyl sumaresinolate (8), m.p. 222 – 223,5°; $[\alpha]_D + 53°$ (CHCl$_3$, c 1.03) (reported m.p. 220 – 221° and $[\alpha]_D + 46.7°$, resp.).[28] The spectroscopic data for the last two compounds have been given elsewhere.[5]

The mass spectral results indicated that fraction 6 (800 mg) in addition to oleanolic acid also contained a compound of molecular weight 284. In order to accomplish a chromatographic separation of these two components, the fraction was first treated with diazomethane in ether for 24 h, and then with acetic anhydride (5 ml) in pyridine (10 ml) for 3 h at 100°. Chromatography on silica gel (hexane-acetone) of the resulting material furnished a methyl oleanolate acetate (155 mg) and a methyl ether of the desired compound, m.w. 298 (90 mg). This was purified further by preparative thin-layer to give an aromatic ester (40 mg) as an oil; ν_{max} (film): 2845, 1710, 1633, 1598, 1582, 1513, 1464, 1455, 1422, 1376, 1260, 1150, 1025, 980, 847, 810, 752, 700 cm^{-1}; m/e (%): 298 (100), 283 (3), 280 (4), 267 (4), 253 (14), 207 (18), 192 (9), 191 (34), 164 (40), 163 (9), 91 (50), 77 (51). The aromatic ester was added to a solution of aqueous KOH (45 %, 1 ml) in ethanol (5 ml) and refluxed for 2 h under N$_2$. Dilution with water and extraction with ether gave benzyl alcohol identified by GLC-MS. The aqueous solution was acidified with dilute H$_2$SO$_4$ (10 %) and extracted with ether to give 3,4-dimethoxycinnamic acid, identified by comparison with a sample prepared from ferulic acid by methylation and subsequent saponification of the methyl ester grouping; m.p. and mixed m.p. 180 – 182°; the IR and mass spectra of the two samples were identical. The original compound found in tolu balsam is thus benzyl ferulate or isoferulate.

Strong acids. Part of the fraction comprising strong acids (1 g) was chromatographed on silica gel (light petroleum – isopropyl ether – acetic acid) to give benzoic acid (549 mg), identified by comparison with an authentic sample (m.p. and mixed m.p. 121 – 122°) and cinnamic acid (298 mg). Traces of a third acid, having an R_F-value on TLC and a mass spectrum identical to those of ferulic acid, were also present.

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