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TRITERPENES OF *PRUNUS SEROTINA* AND *P LUSITANICA**

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Abstract—The leaves of *Prunus serotina* and *P lusitanica* contain a new triterpene, 2α 3α-dihydroxyurs-12-en-28-oic acid, isolated in form of its methyl ester Other triterpenes present in these species are ursolic acid and ursol aldehyde *P lusitanica* also yields friedeline

INTRODUCTION

Prunus serotina Ehrh, originated in North America, is frequently found in Dutch woods and has even developed into a serious problem *P lusitanica* L, the Portuguese cherry laurel, is from the Canary Islands, Southwest Spain, and Portugal Our interest in these species was aroused by a communication[1] which stated that larvae of the silkmoth *Hyalophora cecropia* L, feeding on *P lusitanica* moult prematurely, whereas when *P serotina* was the host plant, normal development took place In the course of our investigation of this phenomenon, we examined the triterpenes of both plants *P serotina* was known to contain ursolic acid,[2] but no other triterpenes from these species seem to have bee ported We now report the presence of a new triterpene acid as well as that of some triterpenes of already known structure in both species

RESULTS

The non-saponifiable fraction of the benzene extract of dried leaves of *P lusitanica* were found to contain friedelin (D A friedelane-3-one) but this compound was not detected in *P serotina* The non-saponifiable fractions of both species contain a compound that shows a vivid blue Liebermann–Burchard Reaction (LBR) We were not able to obtain this compound in a sufficiently pure state to allow identification by spectroscopical means However, its behaviour on argentated silica and the fact of its being oxidized to ursolic acid by the silver nitrate–ammonia reagent strongly suggested it to be ursol aldehyde This was proved by a chromatographical comparison of the compound with a synthetic sample of urs-12-en-28-al-3β-ol (**1**) More neutral triterpenes appear to be present in both plants and are currently under investigation

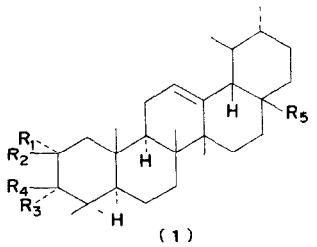
* Part I in the projected series "Constituents of Prunus species"

† Part of this work was published in a thesis "Phytochemical investigations of Prunus lusitanica and Prunus serotina, host plants of Hyalophora cecropia" Utrecht (1972)

[1] STAAL, G B (1967) Report 1967-I from Research group Integrated Control of Insects, Wageningen

[2] POWER, F B and MOORE, CH W (1910) *J Chem Soc* **97**, 1009

TLCs of the acid fractions of both species show an identical picture on detection with LB reagent Both fractions were methylated and chromatographed on silica The main product isolated appeared to be methyl ursolate (**2**), however a new product, methyl 2α,3α-dihydroxyurs-12-en-28-oate (**3**) (colourless needles m p 196 198) was also isolated The reasoning leading to the assignment of this structure to **3** is given below



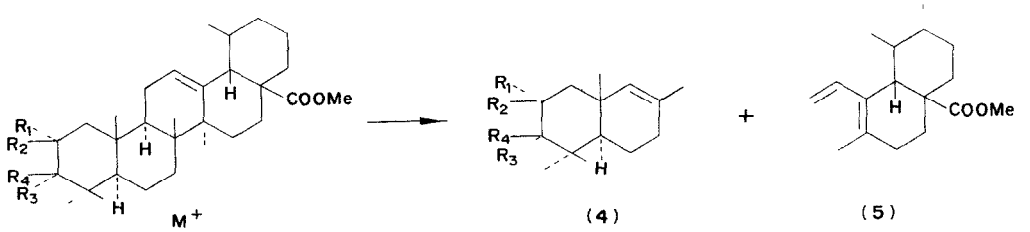
(1)

(1) $R_1 = R_2 = R_3 = H$, $R_4 = OH$, $R_5 = CHO$
(2) $R_1 = R_2 = R_3 = H$, $R_4 = OH$, $R_5 = COOMe$
(3) $R_1 = R_3 = OH$, $R_2 = R_4 = H$, $R_5 = COOMe$
(3a) $R_1 = R_3 = OAc$, $R_2 = R_4 = H$, $R_5 = COOMe$
(6) $R_1 = R_2 = R_3 = H$, $R_4 = OH$, $R_5 = CH_2OH$

High resolution MS of **3** indicates an elementary formula $C_{31}H_{50}O_4$ and gives the characteristic pattern of a Δ^{12}-ursene or a Δ^{12}-oleanene [3] However, the IR spectrum of **3** (in pyridine) shows absorptions at 1390, 1380, 1360 (weak), 1308, 1276 and 1238 cm^{-1}, strongly resembling that reported by Snatzke[4] for compounds with an ursene-type structure The PMR spectrum of **3**, in the range δ 0 75–1 25 (δ in ppm downfield from TMS), shows five singulet methyl signals and two doublets, partly obscured by the singulet peaks, also in agreement with an ursane-type compound, in the PMR spectrum of an oleanene-type compound seven methyl singulets and no doublets would have been observed Also present in the PMR spectrum of **3** is a doublet (J 11Hz) at δ 2 22, attributed by Cheung and Yan[5] to the C-18 proton in a Δ^{12}-ursene compound the corresponding oleanene compound gives rise to an *AB*-quartet at δ 2 8

A broad signal (1 H) at δ 5 22 confirms the presence of a double bond in **3**

The mass spectrum of **3** shows a striking resemblance to that of methyl ursolate In both spectra a base peak at *m/e* 262 appears, thus indicating that rings D and E from methyl ursolate form a part of **3** as well



M^+ (4) (5)

Methyl ursolate $R_1 = R_2 = R_3 = H$, $R_4 = OH$	*m/e* 470	*m/e* 207	*m/e* 262
(3) $R_1 = R_3 = OH$, $R_2 = R_4 = H$	*m/e* 486	*m/e* 223	*m/e* 262

[3] BUDZIKIEWICZ H DJERASSI C and WILLIAMS D H (1964) *Structure Elucidation of Natural Products by Mass Spectrometry* Vol II, p 122 Holden-Day, San Francisco
[4] SNATZKE, G LAMPERT F and TSCHESCHE R (1962) *Tetrahedron* **18**, 1417
[5] CHEUNG T and YAN T C (1972) *Australian J Chem* **25**, 2003

The presence of the COOMe group is confirmed by the IR (carbonyl absorption at 1725 cm^{-1}), PMR (singulet, 3H, at δ 3 58) and MS (peaks at *m/e* 427 ($M^+ - COOMe$) and 203 (**5** –COOMe)) **3** contains one oxygen atom more than methyl ursolate The appearance of a signal at *m/e* 223 in the MS of **3** and the absence of one at *m/e* 207 shows that the additional oxygen atom is present in ring A or B (see Scheme 1)

A peak at about 3400 cm^{-1} in the IR spectrum of **3** (in KBr) shows the presence of at least one hydroxyl group The MS contains peaks at *m/e* 468 (M^+-H_2O) and *m/e* 450 (M^+-$2H_2O$) In the PMR spectrum a signal at δ 3 39 (1 H, *d*, *J* 3 Hz) and one centered at δ 3 97 (1 H, *m*) can be attributed to the α-protons of two secondary hydroxyl groups The presence of two hydroxyl groups is further confirmed by acetylation of **3**, according to the MS a diacetate (**3a**) is obtained (peaks at *m/e* 570 (M^+), *m/e* 510 (M^+-MeCOOH), and *m/e* 450 (M^+-2 MeCOOH)

The last problem to be solved was the position of the two OH-groups in the rings A and/or B On account of the doublet at δ 3 39 in the PMR spectrum of **3** the two hydroxyl groups should be vicinal They can only be so placed at the positions 1 and 2, 2 and 3, or 6 and 7

In a 6,7-diol the C-6 proton should give rise to a quartet, the signal at δ 3 97 is much more complicated Furthermore, the presence of a OH-group at C-6 gives rise to a peak at *m/e* 302 in the MS[6] (due to dehydration and a retro Diels–Alder reaction), this signal is virtually absent in the MS of **3** Thus the 6,7-diol is excluded

For biogenetic reasons a 2,3-diol is very likely and to be preferred to an 1,2-diol Of the four 2,3-diol systems possible three can be ruled out by examining the IR spectrum of **3** (in CCl_4, 0 005 M) in this spectrum three peaks appear, respectively at 3643, 3623 and 3580 cm^{-1}, with increasing intensity Tschesche *et al*[7] have shown that, of the oleanene diols, only the 2β,3β-diol structure gives rise to three absorptions in this range

Recently, Cheung and Yan[8] concluded from PMR data that the structures for the two *cis*-2,3-diols of the oleanene series as synthesized by Djerassi *et al*[9] and used by Tschesche *et al*[7] for their experiment should be interchanged Consequently **3** should possess the 2α,3α-diol configuration This conclusion is confirmed by comparing the chemical shifts of the singulet methyl groups in the PMR spectra of the four possible methyl-urs-12-en-28-oate-2,3-diols, calculated according to Cheung,[10] with the shifts of the methyl groups of **3** and those of the two ursene diols synthesized by us, the, 2α,3α-diol and the 2β,3β-diol (Table 1)

The two *cis* ursene diols were synthesized by dehydration of methyl ursolate followed by oxidation with OsO_4 The two diols were separated by repeated chromatography on alumina From Table 1 it can be seen that both the 2α,3α-diol and the 2α-3β-diol can give rise to the methyl singlet shifts of **3**, however, the 2α,3β-diol has been ruled out on account of the IR spectral data obtained for **3**, the shifts of **3** and those of the synthesized 2α,3α-diol agree very well The structure of the 2β,3β ursene diol is fully confirmed by its IR-spectrum (in CCl_4) and its PMR spectrum It should be noted that the IR and PMR spectral data further reduce the (already small) possibility of **3** being a 1,2-ursene diol

[6] PINHAS, H (1969) *Bull Soc Chim Fr* 3592
[7] TSCHESCHE, R, HENCKEL, E and SNATZKE, G (1964) *Liebigs Ann Chem.* **676,** 175
[8] CHEUNG, H T and YAN, T C (1970) *J Chem Soc* D, 369
[9] DJERASSI, C, THOMAS, D B, LIVINGSTON, A L and THOMPSON, C R (1957) *J Am Chem. Soc* **79,** 5292
[10] CHEUNG, H T and WILLIAMSON, D G (1969) *Tetrahedron* **25,** 119

In conclusion, it can now be stated that the compound (**3**) we have isolated, is methyl 2α,3α-dihydroxyurs-12-en-28-oate Accordingly the acid present in *P serotina* and *P lusitanica* is 2α,3α-dihydroxyurs-12-en-28-oic acid

TABLE 1 CHEMICAL SHIFTS* OF THE SINGULET METHYL GROUPS IN METHYL URS-12-EN-28-OATE-2,3-DIOLS

	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}
2α,3α OH†	0 98	0 875	0 94	0 73	1 075
2α,3β OH†	1 00	0 81	0 94	0 75	1 07
2β,3α OH†	0 975	1 07	1 23	0 76	1 075
2β,3β OH†	1 02	1 00	1 23	0 775	1 075
3‡	1 00	0 84	0 94	0 72	1 07
2α,3α OH§	1 01	0 85	0 96	0 73	1 10
2β,3β OH§	0 99	0 99	1 22	0 75	1 05

* In ppm downfield from TMS

† Calculated according to Cheung[8 10] for the four possible methyl urs-12-en-28-oate-2,3-diols

‡ Found for **3**

§ Found for the synthesized methyl urs-12-en-28-oate-2,3-diols

DISCUSSION

Friedelin has previously been reported as a constituent of *Prunus turfosa*[11] and *P nepalensis*[12] and has now been identified in *P lusitanica* However, its absence in *P serotina* underlines Sainsbury's statement that any taxonomic conclusion is precluded with respect to friedeline

Hitherto, ursolic acid was found in all the *Prunus* species investigated [12,13] Therefore, its occurrence in *P lusitanica* is not surprising The co-occurrence of ursol aldehyde and ursolic acid suggests a biosynthetic sequence α-amyrine → uvaol → ursol aldehyde → ursolic acid As the presence of the corresponding compounds in the lupane series in *Alangium lamarekii* Thw [14] has been reported, it seems interesting to investigate the neutral triterpene alcohols of *P lusitanica* and *P serotina* It should be noted that ursol aldehyde was demonstrated to be present in some Dipterocarpaceae[15 16] but only in a few cases in co-occurrence with ursolic acid

Another question arises from the presence of 2α,3α-dihydroxyurs-12-en-28-oic acid in *P lusitanica* and *P serotina* is it synthesized from the main triterpene acid present i e ursolic acid, and if so, how does the biosynthesis take place? We are now looking for possible triterpene acid intermediates to answer these questions

EXPERIMENTAL

M ps are uncorrected IR spectra were recorded on a Beckman IR 8 or a Perkin Elmer 457 double beam spectrometer PMR spectra were recorded on a Varian HA 100 in $CDCl_3$ with TMS as an internal standard MS were recorded on an AEI MS 902 at 70 eV

Non-saponifiable material Prunus lusitanica 1 0 kg of dried leaves (collected in June 1969) were extracted with hot C_6H_6 The solvent was removed and the residue dissolved in a mixture of 3 l MeOH 300 ml H_2O and

[11] SAINSBURY, M (1970) *Phytochemistry* **9**, 2209

[12] BARUA A K and MAITI P C (1957) *Sci Cult* **23**, 155

[13] LE MEN K and POURRAT H (1955) *Ann Pharm Fr* **13**, 169

[14] PAKRASHI, S C, BHATTACHARYYA J, MOOKERJEE, S and SAMANTA, T B (1968) *Phytochemistry* **7**, 461

[15] BISSET, N G, CHAVANEL, V, LANTZ, J P and WOLFF R E (1971) *Phytochemistry* **10**, 2451

[16] BISSET, N G DIAZ, M A EHRET, C and OURISSON G (1967) *Phytochemistry* **6**, 1395

92 4 g KOH and refluxed under N_2 for 2 hr The non-saponifiable fraction (NSF) was obtained after removal of MeOH and addition of H_2O followed by extraction with Et_2O The acid fraction was obtained by acidifying the H_2O layer and extraction with Et_2O The Et_2O extract containing the NSF was dried, the solvent removed and the fraction redissolved in hexane From this soln on standing, a solid precipitated that was recrystallized from MeOH–$CHCl_3$ (1 1) to give friedelin (0 5 g), identified by comparing its IR, PMR and MS with those of an authentic sample The hexane soln was chromatographed on silica Elution with C_6H_6 gave a mixture of triterpenes Further elution with C_6H_6–Et_2O (19 1) afforded a substance that showed a vivid blue LBR Rechromatography on silica with hexane–EtOAc (4 1) yielded 300 mg of an oil (**1**) Chromatographying this oil on silica impregnated with 5% $AgNO_3$ gave a crystalline product on elution with Me_2CO This was treated with dil NH_3, subsequent extraction with Et_2O gave a mixture of products in which only a small amount of **1** was present One of the main products was shown to be identical with ursolic acid by TLC

Prunus serotina 1 0 Kg of fresh leaves (collected in June 1969) was dried and treated in the same way as described for *P lusitanica* No friedelin could be detected in the NSF by TLC **1** was present in an estimated amount of 30 mg

Reduction of methyl ursolate 60 mg methyl ursolate was reduced with 100 mg $LiAlH_4$ in Et_2O to give 58 mg urs-12-en-3β,28-diol (uvaol) (**6**), m p 229–230° after recrystallization from MeOH (lit[17] 232–233°)

Oxydation of uvaol to ursol aldehyde **6** was stirred for 1 hr with freshly prepared MnO_2 in hexane at 20° The resulting mixture of products was chromatographed on silica with $CHCl_3$ to give a few mg of a pure compound (**7**) [IR in $CHCl_3$ 3565 cm^{-1} (OH) and 1726 cm^{-1} (aldehyde)]

TLC of **7** *and* **1** **7** and **1** behaved identically in the following TLC systems SiO_2–G, eluentia $CHCl_3$, Et_2O, $CHCl_3$–Me_2CO (9 1) C_6H_6–EtOAc (1 2), C_6H_6–Et_2O (9 1) SiO_2–G + 5% $AgNO_3$, eluentia C_6H_6–Et_2O (9 1) hexane–EtOAc (4 1), C_6H_6–EtOAc (1 2) Al_2O_3 neutral, eluentia C_6H_6, hexane–EtOAc (19 1), hexane–EtOAc (4 1), $CHCl_3$–Me_2CO (9 1), $CHCl_3$–MeOH (9 1)

Acid fractions The acid fractions of both species were treated with CH_2N_2 in Et_2O, and the reaction products were examined by TLC SiO_2–G, $CHCl_3$–Me_2CO (9 1) detection with LBR R_f 0 56 (red brown), 0 37 (pink), 0 21 (pink), 0 15 (pink), 0 11 (pink) and 0 02 (purple) The reaction products were chromatographed on silica Elution with $CHCl_3$ gave methyl ursolate, identified by its IR, PMR, and MS From *P lusitanica* 1 5 g was obtained an oily product that could be purified by rechromatography on silica with Et_2O This gave 230 mg (resp 35 mg) of pure **3**, m p after recrystallization from MeOH–H_2O (4 1) 196–198°, α_D^{20} + 52° (c 1 2, $CHCl_3$), IR (1% in KBr) 3430 1032 and 990 cm^{-1} (OH), 1722 (CO), IR (0 5% in pyridine) 1390, 1380, 1360 (w), 1308, 1276 and 1236 cm^{-1} (ursane skeleton), IR (0 005 M in CCl_4) 3643, 3623 and 3579 5 cm^{-1} (2α,3α-diol) PMR δ 5 22 (1 H, broad, $\geq$C=C<u>H</u>–CH_2), 3 97 (1 H, *m*, –CH_2–C<u>H</u>OH–C), 3 58 (3 H, *s*, –COOC<u>H_3</u>), 3 39 (1 H, *d*, *J* 3 Hz, –CHOH–C<u>H</u>OH–C), 2 22 (1 H, *d*, *J* 11 Hz), and singulets of Me-groups at 1 07, 1 00, 0 94, 0 84 and 0 72 MS M^+ 486 (Found 486 3690, Required for $C_{31}H_{50}O_4$ 486 3709), *m/e* 471 (M^+-CH_3), 468 (M^+-H_2O), 453 (M^+-Me-H_2O), 435 (M^+-Me-2H_2O) 426 (M^+-MeCOOH), 262, 249, 223, 203, 189, 133 and 119 Acetylation of **3** was with pyridine–HOAc (1 1) and leaving the mixture overnight Solvent was evaporated and the residue chromatographed on silica with $CHCl_3$ to give pure **3**-diacetate (MS M^+ 570, *m/e* 510 (M^+-MeCOOH) and 450 (M^+-2 MeCOOH)

Dehydration of methyl ursolate 1 0 g methyl ursolate in 15 ml pyridine and 1 5 ml mesylchloride was left at room temp for 24 hr H_2O was added, the mixture acidified with H_2SO_4 and extracted with Et_2O This gave 1 1 g crude mesylate that was recrystallized 2 × from EtOH to yield 780 mg colourless needles m p 120–124° The mesylate was heated in 75 ml pyridine for 22 hr at 130° The solvent was evaporated and the residue was shown by TLC to consist of 2 products (SiO_2–G + 5% $AgNO_3$, hexane–C_6H_6 (1 1), R_f 0 45 and 0 35) Separation was performed by chromatography on silica to yield 470 mg of methyl urs-2,12-dien-28-oate (**8**) (R_f 0 45). Recrystallization from MeOH–$CHCl_3$ gave colourless needles m p 192–194° The PMR and MS were in accordance with these structure

OsO_4-oxydation of **8** 108 mg **8** and 750 mg OsO_4 in dioxane were left for 12 days at room temp After passing H_2S through the soln this was filtered and the filtrate evaporated to dryness The residue was chromatographed several times on acidic Al_2O_3 with Et_2O–MeOH (19 1) to give 30 mg of pure diol A (R_f 0 55) and 27 mg of pure diol B (R_f 0 46) The latter was chromatographically and spectroscopically identical with compound **3** from both *Prunus* species

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