Phytochemistry, Vol 23, No 7, pp 1461–1463, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

LATHYRANE TYPE DITERPENE ESTERS FROM *EUPHORBIA LATHYRIS*

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(*Received* 18 *November* 1983)

Key Word Index—*Euphorbia lathyris*, Euphorbiaceae, lathyrane, diterpene esters, 17-hydroxyjolkinol, 17-hydroxyisolathyrol

Abstract—By reversed phase HPLC 'ester L_7', previously isolated among several macrocyclic lathyrol derivatives from seeds of *Euphorbia lathyris*, was resolved into two compounds, L_{7a} and L_{7b} Their structures were elucidated by spectroscopic analysis and transesterification reactions as 15,17-di-*O*-acetyl-3-*O*-cinnamoyl-17-hydroxyjolkinol and 5,15,17-tri-*O*-acetyl-3-*O*-benzoyl-17-hydroxyisolathyrol, respectively

INTRODUCTION

Euphorbia lathyris L ('caper spurge') has recently been discussed as a source of biomass and fuel [1, 2] Chemical investigations of the heptane-extractables of the dried plant [3] and of the latex [4] afforded almost exclusively triterpenoids and sterols Moreover, the acetone extract of the latex and the ether extract of the seeds of *E lathyris* revealed tetracyclic diterpene esters of the ingenane type, e g factor L_5, exhibiting irritant and tumour promoting activity in mouse skin [5] A number of structurally related non-irritant diterpene esters of the tricyclic lathyrane type were isolated from seeds (L_1–L_3, L_7, L_8) [6–8], from roots (jolkinol B) and from callus cultures (jolkinols A and A') [9] With the exception of 'ester L_7' all structures were elucidated Recently, L_7 was resolved into two compounds, L_{7a} and L_{7b} Their structures have now been derived on the basis of spectral data and transesterification reactions

RESULTS AND DISCUSSION

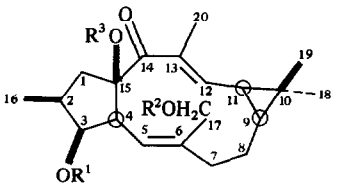
'Ester L_7' may be isolated either by preparative TLC of a fraction obtained after Craig distribution of the neutral fraction (see ref [5]) or after column chromatography of the neutral fraction of the seed oil of *E lathyris* By reversed phase (octadecylsilane) high pressure liquid chromatography it was further separated into two compounds L_{7a} and L_{7b}

17-*Hydroxyjolkinol** *and its esters*

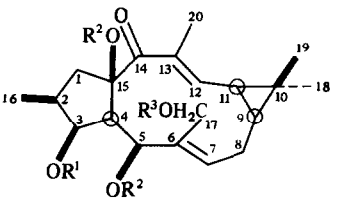
The ^{1}H NMR and mass spectra of L_{7a} (**1a**) (M^+ at m/z 548) indicated the presence of a triester of a diterpene alcohol $C_{20}H_{30}O_4$ esterified with two acetic acid and one cinnamic acid moieties Taking into account the UV absorption of cinnamic acid with λ_{max} 272 nm (ε12 000) [12], the UV extinction at 277 nm (33 500) is very characteristic for the β-cyclopropyl enone system in all lathyrol and jolkinol derivatives [6–10] and in crotonitenone [13] Indeed, decoupling experiments proved the vicinity of the cyclopropane proton H-11 (δ1 44) with the olefinic proton H-12, which showed a long range coupling with the methyl protons at Me-20 Furthermore, like in all other lathyrane type diterpenoids proton H_a-1 appeared as a *dd* at very low field (δ3 60) By decoupling experiments, the sequence H_a-1, H-2 (Me-16), H-3, H-4 and H-5 was deduced However, in contrast to the lathyrol triesters, H-5 did not appear at *ca* δ6 2 [8] but rather at δ5 69 The same chemical shift and coupling constant was found for H-5 in jolkinol D [11], a lathyrane derivative with a 5,6-double bond The preparation of the parent alcohol **1b** (see below) without diamagnetic shift of H-5 furnished proof of its olefinic character In contrast to jolkinol D, in the ^{1}H NMR spectrum of L_{7a} the signal (δ1 46) for the vinylic methyl group Me-17 was missing and an AB system of two protons at δ4 28 was present This suggested hydroxylation of the methyl group resulting in a 17-acyloxy group with two allylic protons at H_2-17 Their signal was diamagnetically shifted upon transesterification, corresponding to a free hydroxyl group (see below) Thus, compound L_{7a} represents the triester **1a** of 17-hydroxyjolkinol (**1b**)

The positions of the acid moieties in L_{7a} were deduced from transesterification with sodium methoxide in methanol which afforded the four reaction products **1b**–**1e** From mass (M^+ at m/z 334) and ^{1}H NMR spectra of **1b** (diamagnetic shifts of protons H-3 and H_2-17 and no signals for acid moieties) the presence of the parent alcohol 17-hydroxyjolkinol was indicated In **1c** (M^+ at m/z 506) an acetyl group had been removed from the C-17 position as indicated by the diamagnetic shift of H_2-17 (0 33 ppm) and by the signal of only one acetyl group In the ^{1}H NMR spectrum of **1d** (M^+ at m/z 464), both acetyl groups present in **1a** were seen to be missing Since the signal for H-3 was not shifted, the position of the cinnamoyl moiety at C-3 was established Compound **1e** (M^+ at m/z 376) was a monoacetate In its ^{1}H NMR spectrum the signals for H-3 and H_2-17 were diamagnetically shifted compared to those in **1a**, proving the presence of a 15-acetate

*The nomenclature recently proposed [10] has been adopted, i e the name jolkinol for the diterpene moiety of jolkinol D [11] and the name isolathyrol for 6,7-dehydro-6,17-dihydrolathyrol



1a R^1 = cinnamoyl, $R^2 = R^3$ = acetyl
1b $R^1 = R^2 = R^3$ = H
1c R^1 = cinnamoyl, R^2 = H, R^3 = acetyl
1d R^1 = cinnamoyl, $R^2 = R^3$ = H
1e $R^1 = R^2$ = H, R^3 = acetyl



2a R^1 = benzoyl, $R^2 = R^3$ = acetyl
2b $R^1 = R^2 = R^3$ = H
2c R^1 = benzoyl, R^2 = acetyl, R^3 = H

From the above reaction products the structure of L_{7a} is unambiguously determined as 15,17-di-*O*-acetyl-3-*O*-cinnamoyl-17-hydroxyjolkinol (**1a**) In compounds **1b** and **1d** with a free 15-hydroxyl group the proton at H-12 is paramagnetically shifted from δ6 6 to *ca* δ7 4 This was already observed for derivatives of jolkinol-6β,7β-oxide and of isolathyrol [10]

17-Hydroxyisolathyrol and its esters

The ^{1}H NMR and mass spectra of L_{7b} (**2a**) (M^+ at *m/z* 580) suggested the presence of a tetraester of a diterpene parent alcohol ($C_{20}H_{30}O_5$) esterified with three acetic acids and with benzoic acid Again, UV absorption at 273 nm (ε12 900) indicated the presence of the β-cyclopropyl enone system which was supported by ^{1}H NMR decoupling experiments From the latter experiments the sequence H_a-1, H-2 (Me-16), H-3, H-4 and H-5 was deduced to be present as in other lathyrol derivatives As in the lathyrol compounds, the signals for H-3 and H-5 appeared at δ5 9 and 6 4, respectively, and these were diamagnetically shifted upon transesterification of L_{7b} proving acyloxy groups were present at the C-3 and C-5 positions

The presence of a further acyloxy group at C-17, as in compound L_{7a}, was indicated by a singlet at δ4 5 corresponding to two protons An additional doublet of doublets appeared at δ5 69 and remained unchanged after transesterification (see below) This indicated the presence of a second double bond in the lathyrane skeleton Since the chemical shift of 17-H_2 was characteristic for its allylic position, the additional double bond must be positioned between C-6 and C-7 furnishing an olefinic proton at H-7 as a doublet of doublets Hence, compound L_{7b} represents the tetraester **2a** of 17-hydroxyisolathyrol (**2b**, for nomenclature, see earlier footnote)

The precise positions of the acid moieties in L_{7b} could not be elucidated from a transesterification reaction which afforded the products **2b** and **2c** The ^{1}H NMR spectral data of **2b** (M^+ at *m/z* 350) were very similar to those of isolathyrol [10] The only difference was the absence of the signal of a vinylic methyl group and the appearance of an AB system for the H_2-17 hydroxymethylene protons at δ4 12 Hence, **2b** represents the diterpene alcohol 17-hydroxyisolathyrol Compared to **2a**, in the ^{1}H NMR spectrum of **2c** (M^+ at *m/z* 538) the signal for one acetyl group was missing Since the signal for H_2-17 was diamagnetically shifted to δ4 07, selective cleavage of an acetyl group from C-17 position is proved Thus, one of the hydroxyl groups at C-3, C-5 or C-15 may be esterified with benzoic acid

The comparison of the ^{1}H NMR data of **2a** with those of lathyrol derivatives may tentatively lead to a proposal for the position of the acid moieties In 3,5-di-*O*-acetyllathyrol [8] the signal of H-3 appears at δ5 55 and in 3,5-di-*O*-acetylisolathyrol [10] it is located at δ5 70 Hence, the chemical shift of H-3 at δ5 90 in the spectrum of **2a** may indicate the benzoyl group is at the C-3 position, i e compound L_{7b} is represented as 5,15,17-tri-*O*-acetyl-3-*O*-benzoyl-17-hydroxyisolathyrol (**2a**)

The occurrence of esters of lathyrol, 6,17-epoxylathyrol, 7-hydroxylathyrol, 17-hydroxyisolathyrol and 17-hydroxyjolkinol in seeds of *E lathyris* as well as of jolkinol B in roots and of jolkinol A and A' in callus cultures indicates a close biogenetic relationship of this class of macrocyclic diterpenes [9] Their possible role as precursors of the irritant and tumour promoting tetracyclic ingenol and phorbol derivatives has already been suggested [14]

EXPERIMENTAL

Spectra Mass spectra were measured at 100 eV UV spectra were obtained in MeOH solns 90 MHz ^{1}H NMR spectra were recorded in $CDCl_3 + D_2O$ solns with TMS as internal standard

Material Ester L_7 (R_f 0 2 in Et_2O–petrol, 1 1) was obtained as described previously [5] It may also be isolated by column chromatography of the neutral fraction of the seed oil of *E lathyris* [5] with petrol–Et_2O (2 1) (yield 0 25% of the seed oil) The resinous L_7 (100 mg) was further separated by HPLC (reversed phase, Lichrosorb RP-18, 10 μm, MeOH–H_2O, 4 1, 115 bar) into two compounds L_{7a} (**1a**, 22 mg, R_f 0 14 in MeOH–H_2O, 4 1, on Merck RP-18 plates, staining light brown with vanillin-sulphuric acid) and L_{7b} (**2a**, 54 mg, R_f 0 24, staining dark brown)

15,17-*Di-O-acetyl-3-O-cinnamoyl-17-hydroxyjolkinol* (**1a**) MS *m/z* (rel int) 548 2761 (M^+, 8 5, calculated for $C_{33}H_{40}O_7$ 548 2774), 533 (1), 505 (30), 489 (10), 488 (14), 446 (6), 445 (5), 428 (9), 417 (4), 400 (4), 358 (10), 357 (11), 340 (29), 298 (56), 297 (44), 131 (100), IR ν_{max}^{KBr} cm^{-1} 1735, 1708 (CO), 1640, 1610 (C=C), UV λ_{max}^{MeOH} (ε) nm 277 (33 500), 223 (17 000), 217 (19 800), ^{1}H NMR δ6 60 (*br d*, J = 11 Hz, H-12), 5 69 (*d*, J = 11 Hz, H-5), 5 44 (*t*, J = 3 5 Hz, H-3), 4 28 ± 0 12 (AB, J_{AB} = 13 Hz, H_2-17), 3 60 (*dd*, J_1 = 14 Hz, J_2 = 8 Hz, H_a-1), 2 80 (*dd*, J_1 = 11 Hz, J_2 = 3 5 Hz, H-4), 1 87 (*br s*, Me-20), 1 16 (*s*) and 1 05 (*s*, Me-18 and Me-19), 0 98 (*d*, J = 7 Hz, Me-16), cinnamoate 7 76 (*d*, J = 16 Hz), 6 50 (*d*, J = 16 Hz), 7 3–7 6 (5 aromatic H), two acetates 2 09 (*s*) and 2 02 (*s*) The results of decoupling experiments are shown in Table 1

Transesterification of **1a** Compound **1a** (20 mg) was treated

Table 1 ^{1}H NMR decoupling experiments with compound **1a**

Irradiated at	Observed at	Change of multiplicity
1 44 (H-11)	6 60 (H-12)	$d \rightarrow s$
6 60 (H-12)	1 87 (Me-20)	$br\,s \rightarrow s$
2 25 (H-2)	0 98 (Me-16)	$d \rightarrow s$
	5 44 (H-3)	$t \rightarrow d$ (J = 3 5 Hz)
	3 60 (H_a-1)	$dd \rightarrow d$ (J = 14 Hz)
2 80 (H-4)	5 69 (H-5)	$d \rightarrow s$
	5 44 (H-3)	$t \rightarrow d$ (J = 3 5 Hz)
5 69 (H-5)	2 80 (H-4)	$dd \rightarrow d$ (J = 3 5 Hz)
7 76 (olef H)	6 50 (olef H)	$d \rightarrow s$

Table 2 ^{1}H NMR decoupling experiments with compound **2a**

Irradiated at	Observed at	Change of multiplicity
1 50 (H-11)	6 70 (H-12)	$d \rightarrow s$
6 70 (H-12)	1 80 (Me-20)	$d \rightarrow s$
2 35 (H-2)	5 90 (H-3)	$t \rightarrow d$ (J = 3 5 Hz)
	0 96 (Me-16)	$d \rightarrow s$
	3 48 (H_a-1)	$dd \rightarrow d$ (J = 14 Hz)
2 81 (H-4)	5 90 (H-3)	$t \rightarrow d$ (J = 3 5 Hz)
	6 40 (H-5)	$d \rightarrow s$
6 40 (H-5)	2 80 (H-4)	$dd \rightarrow d$ (J = 3 5 Hz)
5 60 (H-3)	2 80 (H-4)	$dd \rightarrow d$ (J = 8 Hz)
2 35 (H_a-8)	5 69 (H-7)	$dd \rightarrow d$ (J = 12 Hz)

with 0 1 M NaOMe–MeOH (2 ml) for 3 hr The reaction mixture was worked up by adding phosphate buffer, pH 6 8, evaporating the MeOH and extracting the aq phase with EtOAc Prep TLC in Et_2O–petrol (2 1), followed by a second development in Et_2O–petrol (3 1) yielded the reaction products **1b** (4 5 mg, R_f 0 14), **1c** (1 5 mg, R_f 0 20), **1d** (2 mg, R_f 0 25) and **1e** (0 6 mg, R_f 0 06)

17-*Hydroxyjolkinol* (**1b**) MS m/z 334 $[M]^+$, UV λ_{max}^{MeOH} (ε) nm 278 (12 900), ^{1}H NMR δ 7 4 (*dd*, J_1 = 11 Hz, J_2 = 1 Hz, H-12), 5 9 (*d*, J = 11 Hz, H-5), 4 02 (*t*, J = 3 5 Hz, H-3), 3 77 ± 0 12 (AB, J_{AB} = 13 Hz, H_2-17), 3 47 (*dd*, J_1 = 14 Hz, J_2 = 9 Hz, H_a-1), 2 44 (*dd*, J_1 = 11 Hz, J_2 = 3 5 Hz, H-4), 1 84 (*d*, J = 1 Hz, Me-20), 1 21 (*s*) and 1 12 (*s*, Me-18 and Me-19), 1 16 (*d*, J = 7 Hz, Me-16)

15-O-*Acetyl*-3-O-*cinnamoyl*-17-*hydroxyjolkinol* (**1c**) MS m/z 506 $[M]^+$, IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1} 3600 (OH), UV λ_{max}^{MeOH} (ε) nm 277 (23 800), ^{1}H NMR data at variance with those in the spectrum of **1a** δ 3 95 (AB, J = 12 Hz, H_2-17), 2 08 (*s*, acetate)

3-O-*Cinnamoyl*-17-*hydroxyjolkinol* (**1d**) MS m/z 464 $[M]^+$, ^{1}H NMR data at variance with those in the spectrum of **1a** δ 7 4–7 6 (*m*, H-12, superimposed with aromatic H of cinnamic acid), 5 56 (*d*, J = 11 Hz, H-5), 5 48 (*t*, J = 3 5 Hz, H-3), 3 84 ± 0 12 (AB, J_{AB} = 12 Hz, H_2-17), no signals for acetates

15-O-*Acetyl*-17-*hydroxyjolkinol* (**1e**) MS m/z 376 $[M]^+$, ^{1}H NMR δ 6 57 (*dd*, J_1 = 11 Hz, J_2 = 1 Hz, H-12), 5 92 (*d*, J = 11 Hz, H-5), 3 98 (*t*, J = 3 5 Hz, H-3), 3 85 ± 0 12 (AB, J_{AB} = 12 Hz, H_2-17), 3 55 (*dd*, J_1 = 14 Hz, J_2 = 8 Hz, H_a-1), 2 57 (*dd*, J_1 = 11 Hz, J_2 = 3 5 Hz, H-4), 1 87 (*d*, J = 1 Hz, Me-20), 2 07 (*s*, acetate)

5,15,17-*Tri*-O-*acetyl*-3-O-*benzoyl*-17-*hydroxyisolathyrol* (**2a**) MS m/z (rel int) 580 $[M]^+$ (48), 565 (0 6), 537 (8), 521 (35), 478 (12), 477 (15), 461 (10), 460 (16), 418 (21), 417 (34), 400 (8), 373 (10), 356 (15), 355 (15), 349 (9), 339 (15), 338 (19), 313 (31), 296 (73), 295 (80), 105 (100), IR ν_{max}^{KBr} cm^{-1} 1735, 1713 (CO), 1648, 1625 (C=C), UV λ_{max}^{MeOH} (ε) nm 273 (12 900), 229 (14 400), ^{1}H NMR δ 6 70 (*br d*, J = 11 Hz, H-12), 6 40 (*d*, J = 9 Hz, H-5), 5 90 (*t*, J = 3 5 Hz, H-3), 5 69 (*dd*, J_1 = 12 Hz, J_2 = 4 5 Hz, H-7), 4 50 (*s*, H_2-17), 3 48 (*dd*, J_1 = 14 Hz, J_2 = 8 Hz, H_a-1), 2 81 (*dd*, J_1 = 8 Hz, J_2 = 3 5 Hz, H-4), 1 80 (*d*, J = 1 Hz, Me-20), 1 37 (*s*) and 1 20 (*s*, Me-18 and Me-19), 0 96 (*d*, J = 6 5 Hz, Me-16), benzoate 8 02 (*m*, 2H) and 7 5 (*m*, 3H), three acetates 2 26 (*s*), 2 07 (*s*) and 1 70 (*s*) The results of decoupling experiments are shown in Table 2

Transesterification of **2a** Compound **2a** (20 mg) was reacted with 0 1 M NaOMe–MeOH and worked up in the same manner as compound **1a** Prep TLC in the same solvent systems as above afforded the reaction products **2b** (5 5 mg, R_f 0 10) and **2c** (2 5 mg, R_f 0 22)

17-*Hydroxyisolathyrol* (**2b**) MS m/z 350 $[M]^+$, UV λ_{max}^{MeOH} (ε) nm 278 (10 670), ^{1}H NMR δ 7 56 (*dd*, J_1 = 10 Hz, J_2 = 1 Hz, H-12), 5 47 (*m*, H-7), 5 18 (*d*, J = 9 Hz, H-5), 4 51 (*t*, J = 3 5 Hz, H-3), 4 12 ± 0 15 (AB, J_{AB} = 12 Hz, H_2-17), 3 25 (*dd*, J_1 = 14 Hz, J_2 = 9 Hz, H_a-1), 2 34 (*dd*, J_1 = 9 Hz, J_2 = 3 5 Hz, H-4), 1 73 (*d*, J = 1 Hz, Me-20), 1 30 (*s*) and 1 20 (*s*, Me-18 and Me-19), 1 16 (*d*, J = 7 Hz, Me-16), 1 50 (H-11, detected by decoupling experiments)

5,15-*Di*-O-*acetyl*-3-O-*benzoyl*-17-*hydroxyisolathyrol* (**2c**) MS m/z 538 $[M]^+$, IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1} 3600 (OH), UV λ_{max}^{MeOH} (ε) nm 277 (10 500), ^{1}H NMR data at variance with those in the spectrum of **2a** δ 4 07 (*s*, H_2-17), two acetates 2 27 (*s*) and 1 72 (*s*)

REFERENCES

1 Calvin, M (1977) *Energy Res* **1**, 299
2 Calvin, M, Nemethy, E K, Redenbaugh, K and Otvos, J W (1982) *Experientia* **38**, 18
3 Nemethy, E K, Otvos, J W and Calvin, M (1979) *J Am Oil Chem Soc* **56**, 957
4 Nielsen, P E, Nishimura, J W, Otvos, J W and Calvin, M (1977) *Science* **198**, 942
5 Adolf, W and Hecker, E (1975) *Z Krebsforsch* **84**, 325
6 Adolf, W, Hecker, E, Balmain, A, Lhomme, M F, Nakatani, Y, Ourisson, G, Ponsinet, G, Pryce, R J, Santhanakrishnan, T S, Matyukhina, L G and Saltikova, I A (1970) *Tetrahedron Letters* 2241
7 Narayanan, P, Röhrl, M, Zechmeister, K, Engel, D W, Hoppe, W, Hecker, E and Adolf, W (1971) *Tetrahedron Letters* 1325
8 Adolf, W and Hecker, E (1971) *Experientia* **27**, 1393
9 Adolf, W, Hecker, E and Becker, H (1984) *Planta Med* (in press)
10 Seip, E H and Hecker, E (1983) *Phytochemistry* **22**, 1791
11 Uemura, D, Nobuhara, K, Nakayama, Y, Shizuri, Y and Hirata, Y (1976) *Tetrahedron Letters* 4593
12 Phillips, J P, Dacons, J C and Rice, R G (eds) (1964–1965) *Organic Electronic Spectral Data*, Vol VII, p 209 Wiley-Interscience, New York
13 Burke, B A, Chan, W R, Pascoe, K O, Blount, J F and Manchand, P S (1981) *J Chem Soc Perkin Trans* 1, 2666
14 Adolf, W and Hecker, E (1977) *Isr J Chem* **16**, 75