

INGENOL ESTERS FROM THE PRO-INFLAMMATORY FRACTION OF *EUPHORBIA KAMERUNICA*

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Abstract—A series of unstable mono- and di-esters of the tetracyclic diterpene ingenol were isolated from the pro-inflammatory ether-soluble fraction of the latex of *Euphorbia kamerunica*. The esters were isolated by a neutral process involving column and thin-layer chromatography. The monoesters were identified by spectroscopic methods and hydrolysis reactions as ingenol-3-decanoate, ingenol-3-dodecanoate, ingenol-5-hexadecanoate and ingenol-5-octenoate and the diesters as 20-acetyl-ingenol-3-octenoate and 20-acetyl-ingenol-3-angelate.

INTRODUCTION

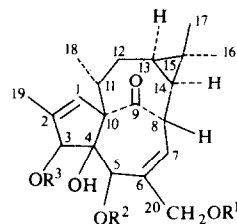
Ingenol is a tetracyclic diterpene isolated initially from *Euphorbia ingens* [1] and its configuration was obtained by X-ray methods [2]. It has been shown by an investigation of several sections of the large genus *Euphorbia* that this parent diterpene is the most widely distributed tetracyclic nucleus of the phorbol type of secondary plant metabolites [3]. Ingenol after hydrolysis of its natural esters and subsequent conversion to its stable acetate can readily be detected from plant extracts [4]. However, few investigations have been made [5] concerning the nature of the esterified diterpenes which occur in plants. This communication describes the isolation of mono- and di-esters of ingenol from the latex of *E. kamerunica*.

RESULTS AND DISCUSSION

The ^1H NMR and mass spectra of esters 1-6 indicated that these compounds were mono- and di-esters of the parent diterpene ingenol (Fig. 1). Compounds 1-4 were monoesters which contained a long chain fatty acid whilst the diesters 5 and 6 additionally exhibited an acetate group. Conversion of these esters to ingenol was achieved using alkali catalysed hydrolysis. Subsequent acetylation afforded the stable acetate 7 which was identical to the previously known ingenol triacetate [1,2,4]. The natural products 1-6 were unstable during extraction and separation. Using unbuffered chromatographic adsorbents it is estimated that over 50% of the compounds would be lost due to adsorbent catalysed conversion to ingenol or its transesterification products. These losses were greatly reduced when TLC plates were prepared from buffered solutions rather than from water. Using neutral separation between 10 and 80 mg of esters were obtained from

500 ml of latex. These yields possibly do not reflect higher levels of ingenol esters in the plant latex itself.

Compounds 1 and 2 were dodecanoate and decanoate esters of ingenol, respectively. From the chemical shifts of the ^1H signals for the protons at C-5 and C-3 in their ^1H NMR spectra these esters were the 3-acyl derivatives [1,5]. Compounds 3 and 4 which were new C-5 esters of ingenol would not separate by either adsorption or partition methods. From the relative sizes of the M^{++} ions in the mass spectrum of this mixture, 4 formed ca 18% and 3 82% of the fraction. Because spectral data was obtained from a mixture of esters the stereochemistry of the double bonds in the acyl groups remain to be



- 1 $\text{R}^1 = \text{R}^2 = \text{H}$, $\text{R}^3 = \text{Dodecanoate}$
- 2 $\text{R}^1 = \text{R}^2 = \text{H}$, $\text{R}^3 = \text{Decanoate}$
- 3 $\text{R}^1 = \text{R}^3 = \text{H}$, $\text{R}^2 = \text{Hexadecanoate}$
- 4 $\text{R}^1 = \text{R}^3 = \text{H}$, $\text{R}^2 = \text{Octenoate}$
- 5 $\text{R}^1 = \text{Acetate}$, $\text{R}^2 = \text{H}$, $\text{R}^3 = \text{Octenoate}$
- 6 $\text{R}^1 = \text{Acetate}$, $\text{R}^2 = \text{H}$, $\text{R}^3 = \text{Angelate}$
- 7 $\text{R}^1 = \text{R}^2 = \text{R}^3 = \text{Acetate}$

Fig. 1. The ingenol esters of *Euphorbia kamerunica*.

established. The variation in the coupling constants for the olefinic protons in the ^1H NMR spectrum indicated that both *cis* and *trans* double bonds were present [6].

Compounds **5** and **6** were new diesters of ingenol and the ^1H NMR spectra of these compounds indicated that an acyl group was present at C-3 of the diterpene nucleus. The higher MW acyl group in **5** was octenoate and in **6** was angelate. The second acyl group of **5** and **6** was acetate. In the ^1H NMR spectra of **5** and **6** the 2H-20 signal had moved downfield from *ca* 4.16 ppm in the spectra of the monoesters to 4.63 ppm, and was exhibited as an AB quartet rather than as a singlet. The second acyl group of **5** and **6** was therefore present at C-20 of ingenol. The diesters were sensitive to alkali and it was not possible to selectively hydrolyse the C-3 or the C-20 acyl positions. However, it is possible to tentatively assign the acetate to C-20 on the basis that C-3 acyl monoesters were isolated from this plant.

EXPERIMENTAL

Extraction. Latex of *E. kamerunica* was collected into MeOH, extracted and the Et₂O soluble latex resin separated by gradient elution CC as previously described [7]. All column fractions were tested for *in vivo* pro-inflammatory action on mammalian skin [8]. Fractions in C₆H₆-EtOAc 3:1, 1:1, 1:3, 1:6 and 1:9 induced erythema in μg quantities.

Monoesters of ingenol. *Ingenol-3-dodecanoate 1:* yield 30 mg from 500 ml of latex. This compound was obtained from the C₆H₆-EtOAc (1:3) fraction. **1** was purified by TLC using kieselguhr G (buffered pH 7) coated with 15% dipropylene glycol and developing with heptane-C₆H₆ (7:3) (*R_f* 0.2). A second partition TLC purification using cyclohexane as solvent (*R_f* 0.1) was also necessary. Final purification was by adsorption TLC using Si gel G (buffered pH 7) and developing with hexane-Et₂O-EtOAc (1:1:1) (*R_f* 0.18). MS (CI isobutane, 190°): *m/z* 530 (*M*⁺, 9%), 329 (19), 313 (49), 122 (100), 83 (100). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3465, 1715, 1615, 1420. ^1H NMR (250 MHz, CDCl₃): δ 6.05 (*m*, H-1, H-7), 5.58 (*s*, H-3), 4.60 (*m*, H-8), 4.15 (*s*, 2H-20), 4.06 (*s*, H-5), 3.64 (OH, exchangeable D₂O), 2.52 (*m*, 2H-12), 2.30 (*m*, -CH₂-CO), 1.8 (*s*, 3H-19), 1.05 (*s*, 3H-16, 3H-17, -(CH₂)₉-), 0.95 (*m*, 3H-18, CH₃-), 0.73 (*m*, H-13, H-14).

Ingenol-3-decanoate 2: yield 80 mg. **2** was isolated from the C₆H₆-EtOAc (1:6) fraction. Further purification involved adsorption TLC as before developing with hexane-Et₂O-EtOAc (1:1:1) (*R_f* 0.18) followed by partition TLC with heptane-C₆H₆ (7:3) (*R_f* 0.18). MS (CI, isobutane, 190°): *m/z* 503 (*M*⁺ + 1, 5%), 502 (*M*⁺, 2), 330 (7), 312 (15), 121 (100), 83 (100). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3465, 1715, 1615. ^1H NMR (250 MHz, CDCl₃): δ 6.05 (*m*, H-1, H-7), 5.58 (*s*, H-3), 4.55 (*m*, H-8), 4.16 (*s*, 2H-20), 4.06 (*s*, H-5), 3.63 (OH, exchangeable D₂O), δ 2.53 (2H-12), 2.29 (*m*, -CH₂-CO), 1.79 (*d*, *J* = 1.1 Hz, 3H-19), 1.05 (*s*, 3H-16, 3H-17, -(CH₂)₇-), 0.96 (*m*, 3H-18, CH₃-), 0.71 (*m*, H-13, H-14).

Ingenol-5-hexedienoate 3 and ingenol-5-octenoate 4: yield 80 mg. Isolated from the C₆H₆-EtOAc (1:9) fraction. The mixture was further purified by adsorption TLC using multiple development with hexane-EtOAc-Et₂O (1:1:1) (*R_f* 0.15) followed by partition TLC using multiple development with heptane-C₆H₆ (7:3) (*R_f* 0.2). MS (CI, isobutane, 190°): *m/z* 472 (*M*⁺, 0.7%), 442 (*M*⁺, 6), 330 (6), 312 (12), 121 (100), 83 (100). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3465, 1715, 1615, 1420. ^1H NMR (250 MHz, CDCl₃): δ 7.84 (*dd*, 1H, olefinic), 6.63 (*t*, 1H, olefinic), 6.35 (*t*, 1H, olefinic), 6.05 (*m*, H-1, H-7, 2H,

olefinic), 5.57 (*d*, 1H, olefinic), 5.31 (*s*, H-5), 4.26 (*m*, H-8), 4.16 (*s*, 2H-20), 4.0 (*s*, H-3), 3.57 (*s*, OH, exchangeable D₂O), 2.54 (*m*, 2H-12), 1.80 (*d*, *J* = 1.4 Hz, 3H-19), 1.08, 1.05 (3H-16, 3H-17, -(CH₂)₇-), 0.93 (*m*, 3H-18, CH₃-), 0.74 (*m*, H-13, H-14).

Ingenol diesters. *20-Acetyl-ingenol-3-octenoate, 5:* yield 30 mg from the C₆H₆-EtOAc (3:1) fraction. **5** was purified by partition TLC using double development with heptane-C₆H₆ (7:3) (*R_f* 0.23). MS (CI, NH₃, 190°): *m/z* 515 (*M*⁺ + 1, 46%), 514 (*M*⁺, 20), 473 (1), 427 (48), 409 (60), 391 (32), 373 (11), 330 (10), 313 (90), 295 (48), 121 (100), 83 (100). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 210 (3.81), 230 (3.45), 257 (2.91), 306 (2.98). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3440, 1740, 1620, 1450. ^1H NMR (250 MHz, CDCl₃): δ 6.13 (*d*, *J* = 2.2 Hz, H-7), 6.06 (*d*, *J* = 1.5 Hz, H-1), 5.97 (*t*, *J* = 15.1 Hz, 1H, olefinic), 5.63 (*m*, H-3, 1H, olefinic), 4.63 (*q*, *J* = 12.5 Hz, 2H-20), 4.15 (*m*, H-8), 3.89 (*s*, H-5), 3.51 (*s*, OH, exchangeable D₂O), 2.50 (*m*, 2H-12), 2.12 (*m*, -CH₂=CH), 2.07 (*s*, CH₃CO-), 1.81 (*d*, *J* = 1.6 Hz, 3H-19), 1.06 (*s*, -(CH₂)₃-), 0.97, 0.96 (3H-16, 3H-17), 0.87 (*m*, 3H-18, CH₃-), 0.72 (*m*, H-13, H-14).

20-Acetyl-ingenol-3-angelate 6: yield 10 mg from the C₆H₆-EtOAc (1:1) fraction. **6** was purified by partition TLC using a single development with heptane-C₆H₆ (7:3) (*R_f* 0.2). MS (CI, isobutane 190°): *m/z* 472 (*M*⁺, 11%), 372 (25), 330 (36), 312 (100), 294 (78), 121 (100), 83 (100). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 215 (3.89), 259 (3.69). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3460, 1725, 1640, 1450. ^1H NMR (250 MHz, CDCl₃): δ 6.13 (*d*, *J* = 3.3 Hz, H-7), 6.1 (*m*, 1H, angelate), 6.05 (*d*, *J* = 1.5 Hz, H-1), 5.62 (*s*, H-3), 4.63 (*q*, *J* = 13 Hz, 2H-20), 4.14 (*m*, H-8), 3.87 (*s*, H-5), 2.49 (*m*, 2H-12), 2.07 (*s*, CH₃CO-), 1.8 (*m*, 3H-19, 6H, angelate), 1.06 (*s*, 3H-16, 3H-17), 0.93 (*s*, 3H-18), 0.73 (*m*, H-13, H-14).

Alkali catalysed hydrolysis. *Ca* 10 mg of each ester was hydrolysed with 0.5 M KOH in dry MeOH for 1 hr. After addition of H₂O the parent diterpene was recovered by partition into CH₂Cl₂. After removal of solvent the diterpene was acetylated with Ac₂O-pyridine (2:1) at 100° for 1 hr. The acetate was purified by adsorption TLC using hexane-Et₂O-EtOAc (1:1:1) (*R_f* 0.42). Compound **7** exhibited the following spectral data: MS (CI, isobutane, 190°): *m/z* 474 (*M*⁺, 1%), 414 (1), 354 (1), 312 (18), 294 (25), 121 (100), 83 (45); IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3430, 1740, 1705, 1640; ^1H NMR (250 MHz, CDCl₃): δ 6.25 (*d*, *J* = 4.6 Hz, H-7), 6.09 (*d*, *J* = 1.7 Hz, H-1), 5.39 (*br s*, H-3), 4.96 (*s*, H-5), 4.39 (*q*, *J* = 12.1 Hz, 2H-20), 4.23 (*m*, H-8), 2.52 (*m*, 2H-12), 2.22, 2.14, 2.0 (3 \times CH₃CO-), 1.76 (*d*, *J* = 1.5 Hz, 3H-19), 1.09, 1.06 (3H-16, 3H-17), 0.99 (*d*, *J* = 7 Hz, 3H-18), 0.73 (*m*, H-13, H-14); CD, (MeOH), (θ) nm: 225 (-6.34 $\times 10^3$), 254 (+3.04 $\times 10^3$), 296 (+3.17 $\times 10^3$).

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