1 The author is grateful to the Bayero University Research and Higher Degrees Committee for financial support, Dr B.W. Staddon for his valuable assistance, Dr D.E. Games for arranging mass spectral facilities.

2 Insect Pests of Nigerian Crops, Research Bulletin, University of Ife/Ministry of Agriculture, Western Nigeria (1968).
3 Staddon, B.W., Everton, I.J., and Games, D.E., Comp. Biochem. Physiol. *62B* (1979) 259.
4 Brennan, B.M., Chang, F., and Mitchell, W.C., Envir. Ent. *6* (1977) 169.
5 Weatherstone, J., Q. Rev. chem. Soc. *21* (1967) 287.
6 Aldrich, J.A., Blum, M.S., Lloyds, J.A., and Fales, H.M., J. chem. Ecol. *4* (1978) 161.
7 Gilby, A.R., and Waterhouse, D.F., Proc. R. Soc. B *162* (1965) 105.
8 Gilby, A.R., and Waterhouse, D.F., Nature *216* (1967) 90.
9 Calam, D.H., and Youdeowei, A., J. Insect Physiol. *14* (1968) 1147.
10 Darooghegh, H., and Olagbemiro, T.O., Experientia *38* (1982) 421.
11 Games, D.E., and Staddon, B.W., J. Insect Physiol. *19* (1973) 1527.
12 McLeod, J.K., Horse, I., Cable, J., Blake J.D., Baker, J.T., and Smith, D., J. Insect. Physiol. *21* (1975) 1219.
13 Smith, R.M., N. Z. Jl Zool. *3* (1964) 375.
14 Ishiwateri, T., Appl. Ent. Zool. *11* (1976) 38.

0014-4754/84/080807-02$1.50 + 0.20/0

Irritant resiniferonol derivatives from Egyptian *Thymelaea hirsuta* L.

A.M. Rizk, F.M. Hammouda, S.E. Ismail[1], M.M. El-Missiry[1] and F.J. Evans

Pharmaceutical Science Laboratory, National Research Centre, Dokki, Cairo (Egypt), and The Department of Pharmacognosy, The School of Pharmacy, University of London, 29–39 Brunswick Square, London, WC1N 1AX (England), 25 October 1983

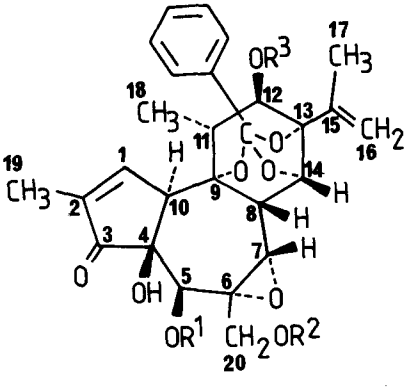
Summary. Two new aromatic derivatives of 5,12-dihydroxy-6,7-epoxy-resiniferonol were isolated from the leaves and twigs of *Thymelea hirsuta* L. (fam. Thymelaeaceae). These compounds were assigned the structures 12-O-cinnamoyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13,14-ortho benzoate **1** and 12-O-heptadecenoyl-5-hydroxy-6,7,-epoxy-resiniferonol-9,13,14-ortho benzoate **2**. Both compounds induced erythema of mouse ears in a dose of 0.1 µg per ear.

The resiniferonol diterpenes are structurally related to the tumor-promoting phorbol-esters from plants of the family Euphorbiaceae[2]. Resiniferonol derivatives were originally isolated from *Daphne* species[3] of the family Thymelaeaceae and are not tumor-promoting agents in 2-stage carcinogenesis tests. However, it has been suggested that they may act at a 3rd stage of carcinogenesis in mammalian systems[4]. Like the phorbol-esters, the resiniferonol-esters induce erythema of skin in low doses[5] but their structure-activity requirements are different, suggesting a different mechanism of action[6].

Thymelaea hirsuta (Thymelaeaceae) is a shrub growing in the Western Egyptian desert and it is known for its toxic activity[7]. 20 kg of dried leaves and twigs were macerated in acetone at room temperature for 1 week. This extract demonstrated pronounced pro-inflammatory activity on the ears of mice using an established assay procedure[5]. After removal of acetone by reduced pressure distillation below 45 °C, the green tar-like residue was dissolved in 40% methanol/water and partitioned against hexane. The hexane fraction contained pigments and lipids and was biologically inactive at doses of 100 µg in the test system above. The methanol/water phase was re-extracted with diethyl ether and the residue from this fraction was shown to induce erythema of mouse ears in a dose of 1 µg per ear. This fraction also exhibited marked cytotoxic activity against TLX/5 mouse lymphoma cells in vitro[8]. The ether fraction was finally freed of green pigment by partition against 0.5% aqueous sodium carbonate solution followed by distilled water until the washings were neutral. Evaporation of the ether solution afforded 15.8 g of a yellow-cream resin. This resin was separated into fractions by means of column chromatography on Florisil using a traditional gradient elution technique[2]. Two biologically active fractions were obtained from the column in yields of about 400 mg.

Fraction 1 was initially purified by means of adsorption preparative-TLC on silica gel G using chloroform/ethyl acetate (2/3) as solvent (R_f 0.10), and further purified by partition preparative-TLC using 20% diethylene glycol on kieselguhr as stationary phase and hexane/butanone (95/5) as mobile phase (R_f 0.22). Final purification was achieved by TLC on silica gel as before using ethyl acetate/acetone (80/20) as solvent (R_f 0.47).

Compound **1** was obtained as a clear glassy resin in a yield of 23 mg. IR ν_{max} (KBr pellet), 3440, 1710, 1660, 1500 cm^{-1}; UV λ_{max} (methanol), 204 ($\varepsilon = 54650$), 215 (shoulder), 280 ($\varepsilon = 42714$), 303 (shoulder) nm; EI-MS (190 °C, 40 e.v., measured values were within 10 ppm of calculated values), $M^{+\cdot}$ m/z 628, $C_{36}H_{36}O_{10}$; CI-MS (190 °C, isobutane), m/z 629 ($M^{+\cdot} + 1$, 50%), 611 (17%), 481 (100%), 419 (7%), 359 (11%), 341 (12%), 323 (11%), 311 (8%), 299 (19%), 253 (20%), 149 (25%), 131 (86%), 123 (43%), 105 (57%); ^{1}H-NMR ($CDCl_3$, 80 MHz, TMS = 0.00 ppm), δ 7.80–7.33 (10H), 7.65 (d, J = 20 Hz, 1H), 7.58 (bs, 1H), 6.37 (d, J = 20 Hz, 1H), 5.18 (s, 1H), 5.05 (s, 2H), 4.95 (d, J = 3.5 Hz, 1H), 4.25 (s, 1H), 3.90 (m,



Compound	R^1	R^2	R^3
1	H	H	OC–CH=CH–⬡
2	H	H	OC–CH=CH–$(CH_2)_{13}CH_3$
3	H	H	H
4	OC–CH_3	OC–CH_3	OC–CH_3

1H), 3.85 (d, J = 8.75 Hz 2H), 3.65 (m, 2H), 2.55 (q, J = 10 Hz, 1H), 1.88 (s, 3H), 1.75 (d, J = 2.5 Hz, 3H), 0.88 (m, 3H), 3.95, 3.58, 2.05 (1H each exchangeable with D_2O ppm). These data were consistent with compound **1** being an aromatic diester of 5,12-dihydroxy-6,7-epoxy-resiniferonol similar to mezerein[3] from *Daphne mazereum* where 1 acyl group was present in the O-acyl form whilst the other was present in the ortho-ester configuration[2].

Fraction 2 from the column was initially purified by TLC on silica gel using chloroform/ethyl acetate (20/30) as solvent (R_f 0.45), and finally by partition TLC as before using cyclohexane/butanone (80/20) as solvent (R_f 0.30).

Compound **2** was obtained as a glassy resin in a yield of 30 mg; the IR spectrum was similar to that of compound **1**; EI-MS (190 °C, 40 e.v.), m/z 748 ($M^{+\cdot}$, 1%), 481 (30%). 358 (15%), 340 (20%), 322 (25%), 317 (22%), 281 (60%), 105 (100%); ^{1}H-NMR ($CDCl_3$, 80 MHz, TMS = 0.00 ppm), δ 7.82–7.10 (5H), 7.58 (bs, 1H), 6.50–5.71 (2H), 5.18 (s, 1H), 5.05 (s, 2H), 4.95 (d, J = 3.5 Hz, 1H), 4.25 (s, 1H), 3.90 (m, 1H), 3.85 (d, J = 8.8 Hz, 2H), 3.65 (m, 2H), 2.55 (q, J = 10 Hz, 1H), 2.10 (m, 2H), 1.88 (s, 3H), 1.75 (m, 3H), 1.20 (bs, 26H), 0.99 (t, 3H), 0.88 (m, 3H) ppm. Compound **2** accordingly was related to **1** but differed from it in that one of the acyl substituents was aliphatic in nature. This derivative therefore belonged to a group of ortho-esters which include Gnididin and Gniditrin previously isolated from *Gnidia lamprantha*[2,9].

The question remained as to which of the acyl substituents in both compound **1** and **2** were in the O-acyl configuration and which were present as the ortho-esters. Compounds **1** and **2** were separately hydrolyzed with 1% sodium methoxide in methanol to produce the common reaction product **3**; EI-MS (170 °C, 40 e.v.), m/z 498 ($M^{+\cdot}$, 1%, $C_{27}H_{30}O_9$); ^{1}H-NMR ($CDCl_3$, 80 MH_z, TMS = 0.00 ppm), δ 7.80–7.30 (5H), 7.56 (bs, 1H), 5.13 (s, 2H), 4.85 (d, J = 3.5 Hz, 1H), 4.25 (s, 1H), 4.20 (s, 1H), 3.90 (s, 1H), 3.85 (d, J = 8.8 Hz, 2H), 3.65 (m, 2H), 2.55 (q, J = 10 Hz, 1H), 1.88 (s, 3H), 1.75 (m, 3H), 0.88 (m, 3H) ppm. This reaction product was 5,12-dihydroxy-6,7-epoxy-resiniferonol-9,13,14-orthobenzoate[3]. A previous X-ray diffraction study of molecular structure and conformation of an O-acyl derivative of this product confirmed its constitution as an ortho-ester[10]. When **3** was acetylated with acetic anhydride/pyridine (2/1) an acetate **4** was produced which from the characteristic shifts of protons on C-5, 12 and 20 together with the appearance of three 3H signals at 2.0-2.1 ppm in this ^{1}H-NMR spectrum confirmed **4** as the 5, 12, 20-triacetate of **3**[2]. On the basis of these data **1** was assigned as 12-O-cinnomoyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13,14-orthobenzoate and **2** was 12-O-heptadecenoyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13,14-orthobenzoate. Compounds **1** and **2** were the major toxic diterpenes from Egyptian *Thymelaea hirsuta* L. These compounds induced erythema in 100% of test animals in a dose of 0.1 µg when tested for pro-inflammatory activity on 2 groups of 36 animals using the mouse-ear model[5].

1 Acknowledgments. S. Ismail and M. El-Missiry are grateful to the Royal Society for a travel and support grant.

2 Evans, F.J., and Taylor, S.E., in: Progress in the Chemistry of Organic Natural Products, vol. 44, p. 1. Eds W. Herz, H. Grisebach and G.W. Kirby. Springer-Verlag, New York 1983.
3 Ronlán, A., and Wickberg, B., Tetrahedron Lett. *1970*, 4261.
4 Blumberg, P.M., Crit. Rev. Toxicol. *8* (1980) 153.
5 Evans, F.J., and Schmidt, R.J., Inflammation *3* (1979) 215.
6 Williamson, E.M., Westwick, J., and Evans, F.J., J. Pharm. Pharmac. *32* (1980) 373.
7 Täckholm, Y., in: Students Flora of Egypt, 2nd edn, p. 360. Cairo University Press, Beirut 1974. *Thymelea hirsuta* was collected near Borg el Arab and authenticated by Professor Batonouny, Department of Botany, University of Cairo.
8 Sayed, M.D., Rizk, A., Hammouda, F.M., El-Missiry, M.M., Williamson, E.M., and Evans, F.J., Experientia *36* (1980) 1206.
9 Kupchan, S.M., Sweeny, J.G., Baxter, R.L., Murae, T., Zimmerly, V.A., and Sickles, B.R., J. Am. chem. Soc. *97* (1975) 672.
10 Nyborg, J., and La Cour, T., Nature *257* (1975) 824.

0014-4754/84/080808-02$1.50 + 0.20/0

Inhibitory effect of ouabain on in vitro and in vivo gastric acid secretion in the frog and the rat

E.S.K. Assem and B.Y.C. Wan[1]

Department of Pharmacology, University College London, Gower Street, London WC1E 6BT (England), 22 July 1983

Summary. The in vitro and in vivo effects of ouabain on gastric acid secretion in the frog and the rat, the 2 species known to have different sensitivity to ouabain, were studied. It was found that ouabain was a potent inhibitor of histamine-stimulated acid secretion in the isolated frog gastric mucosa. Ouabain administered i.v. at dose levels far below the lethal range also produced a marked and significant reduction of histamine-stimulated gastric acid secretion in the anesthetized frogs and rats. It is considered that the inhibitory effect of ouabain on acid secretion could be partly related to its specific antagonizing action on the Na^+-K^+-ATPase in the gastric mucosa.

Ouabain, one of the cardiac glycosides, is a well-known specific inhibitor of the sodium- and potassium- activated adenosine triphosphatase (Na^+-K^+-ATPase). Despite a considerable amount of work carried out to study the mode of action of ouabain on the gastric mucosa, published results concerning the effect of ouabain on gastric acid secretion remain controversal[2-5]. The aim of the present study was to investigate more fully the in vitro and in vivo effect of ouabain on histamine-stimulated gastric acid secretion in the frog. In addition, comparative studies were performed to evaluate the action of ouabain on histamine-stimulated gastric acid secretion in the rat, a species found to be relatively insensitive to ouabain[6].

Materials and methods. Rana temporaria temporaria L., b.wt about 30 g, was used in all experiments on frog gastric acid secretion. For in vitro experiments, the frog gastric mucosa free of muscle layers was mounted between 2 glass chambers. 50 ml of serosal and mucosal solution were used to bathe the serosa and mucosa respectively. The serosal solution contained (mM): NaCl 85.3, KCl 3.4, KH_2PO_4 0.9, $MgSO_4 \cdot 7H_2O$ 0.9, $CaCl_2$ 1.8, $NaHCO_3$ 1.76 and glucose 0.2%. The mucosal solution had the same composition as the serosal solution except that $NaHCO_3$ was replaced by equimolar NaCl. Both solutions were gassed with 100% O_2. The mucosal solution was changed every 30 min. The experiments were performed at room temperature (20–22 °C). Drugs were added to the serosa and expressed as the final concentration in the serosal solution. For whole animal experiments, the frogs were anesthetized by half-immersion in a solution containing 0.1% MS 222 Sandoz