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Milanjilactones A and B, two novel cytotoxic norditerpene dilactones from *Podocarpus milanjianus* Rendle[1]

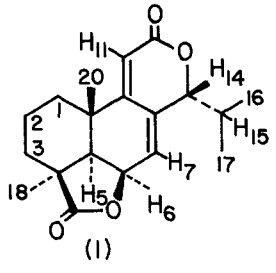
J.A. Hembree, Ching-jer Chang, J.L. McLaughlin and J.M. Cassady[1]

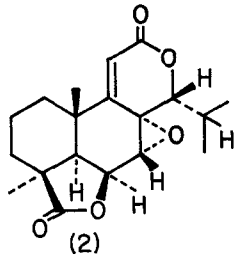
Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette (Indiana 47907, USA), 17 April 1979

Summary. Two new cytotoxic norditerpene dilactones, milanjilactones A (**3**) and B (**4**) have been isolated from the stem bark of *Podocarpus milanjianus* Rendle and characterized, on the basis of spectroscopic evidence, as 1,2-dehydro derivatives of nagilactones F (**1**) and G (**2**).

The genus *Podocarpus* (Taxaceae) is a rich source of terpenic substances from which a number of norditerpene dilactones have been isolated[2]. Earlier we reported that the stem bark of *Podocarpus milanjianus* Rendle, from Kenya, contained 2 norditerpene dilactones (nagilactones F and G, **1** and **2**) which were cytotoxic to 9 KB nasopharynx carcinoma cells[3,4]. Similar activity directed fractionation has now led to the isolation of 2 additional cytotoxic components, milanjilactones A (**3**) ($ED_{50} = 4 \times 10^{\circ}$ μg/ml) and B (**4**) ($ED_{50} = 1 \times 10^{-1}$ μg/ml). The active mixture of **3** and **4** ($ED_{50} = 1 \times 10^{-2}$ μg/ml) was separated from extracts[3] containing **1** and **2** by C-18 reversed-phase silica gel column chromatography. Multiple development preparative-layer chromatography on silica gel then resolved **3** and **4**.

Milanjilactone A (**3**), m.p. 237–238 °C, $C_{19}H_{22}O_5$ (M^+ m/e 330.144), showed UV absorption which supported the


(1)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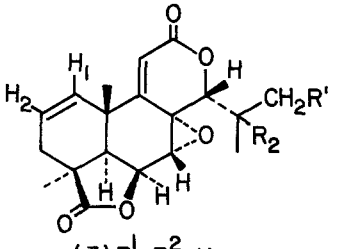
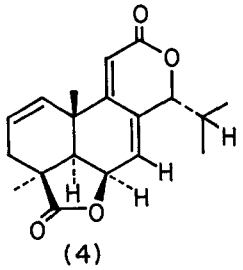

(3) $R^1 = R^2 = H$
(6) $R^1 = SOCH_3$, $R_2 = OH$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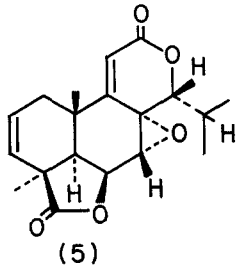
(5)

The PMR-spectra[a] of nagilactone F (**1**), nagilactone G (**2**), milanjilactone A (**3**), milanjilactone B (**4**), podolide[b] (**5**) and podolactone D[c] (**6**)

Compound	H-1	H-2	H-3	H-5	H-6	H-7	H-11	H-14	H-16	H-17	H-18	H-20
1	–	–	–	1.59d (4.7)	5.08td (4.7, 4.7, 1.7)	6.19dt (4.7, 1.7, 1.7)	5.76d (1.7)	4.88q (1.7, 1.7, 1.7)	0.98d[d] (6.8)	1.20d[d] (6.8)	1.34s	1.16s
2	–	–	–	1.85d (4.4)	4.94dd (5.5, 1.5)	3.95d (1.5)	5.96s	4.43d (3.7)	1.10d[d] (8.0)	1.08d[d] (8.0)	1.29s	1.16s
3	(5.80–5.92)		–	2.01d (5)	4.95dd (5, 1.4)	3.96d (1.4)	5.97s	4.34d (3.8)	1.08d[d] (6.7)	1.10d[d] (6.7)	1.35s	1.24s
4[e]	(5.80–6.05m)		–	2.10d (4.7)	5.06td (4.7, 4.7, 1.7)	6.19dt (4.7, 1.7, 1.7)	5.75d (1.7)	4.88q (1.7, 1.7, 1.7)	0.98d[d] (6.8)	1.20d[d] (6.8)	1.38s	1.24s
5	–	(5.80–6.03)		1.85d (5.5)	4.94dd (5.5, 1.5)	3.90d (1.5)	5.97s	4.40d (3.7)	1.06d[d] (7.0)	0.92d[d] (7.0)	1.20s	1.03s
6	5.88d (10.5)	5.75m (10.5, 3.5, < 1)	–	2.05d (5.0)	5.06dd (5.0, 1.3)	5.25d (1.3)	6.19s	4.89s	–	–	1.29s	1.15s

[a] PMR-spectra for compounds **1–5** were obtained in $CDCl_3$ on a Varian FT-80 Spectrometer. Shifts are reported in ppm (δ); J = Hz; d = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, q = quartet, m = multiplet. [b] Authentic sample. [c] Reported[5] in pyridine-d_5. [d] May be reversed. [e] Chemical shifts in pyridine-d_5: H-16 and H-17, 1.01d (6.7), 1.15d (6.7); H-18, 1.38s; H-20, 1.17s.

presence of an α,β-unsaturated δ-lactone grouping at λ_{max}^{EtOH} 220 nm ($\varepsilon = 10{,}000$). Its IR absorption supported this, showing bands at ν_{max} 1765 cm^{-1} (γ-lactone) consistent with related norditerpene lactones[2,5,6]. The PMR-spectrum of **3** closely resembled that of nagilactone G (**2**), podolide (**5**)[7], and podolactone D (**6**)[5]. The multiplet between δ 5.86 and δ 5.92 in (**3**) was assigned to the H-1 and H-2 protons due to a close similarity to shift values reported for H-1 and H-2 of (**6**). Additional support for the olefinic assignment of H-1 and H-2 was obtained from the chemical shift observed for the H-20 protons of (**3**) found at δ 1.24 which closely correlated to that at δ 1.15 for (**6**)[8]. In comparison the signals for the C-20 protons of (**5**) were found further upfield at δ 1.03. The chemical shift value of the C-18 3 proton singlet at δ 1.35 discounted possible positioning of unsaturation between C-2 and C-3 thus establishing its position between C-1 and C-2 as in **6**. TLC on C_{18} reversed-phase silica gel, using methanol: water: acetonitrile (5:3:2) as developing solvent, differentiated the ring A double bond isomers milanjilactone A (R_f 0.44, **3**) and podolide (R_f 0.38, **5**).

2 pairs of 3 proton doublets at δ 1.08 and δ 1.10 indicated the presence of an isopropyl grouping at C-14. Protons H-5, H-6, H-7, H-11 and H-14 were assigned by analogy from their shift values and splitting patterns (table).

Milanjilactone B (**4**), m.p. 220–222 °C, $C_{19}H_{22}O_4$ (M^+ m/e 314.151) showed UV-absorption which supported the presence of a dienolide system identical to that contained in nagilactone F (**1**) at λ_{max}^{EtOH} 257 nm ($\varepsilon = 13{,}000$). Its IR-spectrum was consistent with this showing bands at ν_{max} (KBr) 1685 and 1610 cm^{-1} for the δ-lactone and 1760 cm^{-1} for the γ-lactone. An isopropyl grouping and the 2 quaternary methyl groups were indicated by signals in the PMR at δ 0.98 (3 H, d, 6.8 Hz), 1.20 (3 H, d, 6.8 Hz), δ 1.24 (3 H, s) and δ 1.38 (3 H, s). Further analysis revealed the couplings of H-6 and H-7 and H-14 ($J_{6,7} = J_{6,14} = 1.7$ Hz)[5] analogous to (**1**) in addition to a long range coupling between H-7 and H-11 (J = 1.7 Hz). The chemical shifts for the H-20 and H-18 protons further supported the presence of the C-1, C-2 double bond, with the H-18 proton singlet at δ 1.38 and the H-20 proton signal at δ 1.17 in pyridine-d_5 solution. This latter signal closely correlated with that at δ 1.15 for (**6**)[8]. The stereochemistry of milanjilactone A (**3**) and milanjilactone B (**4**) is proposed based on analogy to nagilactone F (**1**) and podolide (**5**), and is consistent with spectral data.

1 To whom correspondence should be addressed. The authors acknowledge the technical assistance of Ms Teresa K. Lightner. Part 11 in the series 'Potential Antitumor Agents'.

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Involvement of ribonuclease in the interactions of macrophages and fibroblasts in experimental silicosis[1]

Sirpa Aho and E. Kulonen

Department of Medical Chemistry, University of Turku, SF-20520 Turku 52 (Finland), 20 February 1979

Summary. Decreased ribonuclease activity in the supernatant from silica-treated macrophages is associated with the enhanced protein synthesis in granulation-tissue slices incubated in this supernatant, and with the decreased degradation of polysomes in granuloma slices and of polysomes isolated from the granulation tissue. The phagocytized silica particles adsorb ribonuclease and perhaps other proteins and thus remove them from the macrophage supernatant.

The treatment of peritoneal macrophages with SiO_2 liberates from their subcellular particles a soluble agent which stimulates collagen synthesis in slices from experimental granulation tissue[2,3]. We recently found that the SiO_2-released macrophage factor maintains the stability of polysomes in the incubated slices, and that the silica-treated macrophages enhance the cell-free synthesis of proteins by polysomes from experimental granuloma[4]. Several reports[5] have suggested that ribonuclease (RNase) activity plays a key role in the turnover of polysomes. The purpose of this study was to explore whether there is a correlation between the RNase activities of SiO_2-treated macrophage preparations and the synthesis of collagen and other proteins in incubated slices or in polysomes of experimental granulation tissue.

Materials and methods. Unstimulated macrophages were harvested from rats by washing the peritoneal cavity with 0.9% NaCl solution containing heparin and the 7000/500 × g sediment prepared as described earlier[3]. The sediment from 4–5 × 10^6 macrophages was suspended in 1 ml of the incubation medium and divided into 2 portions to be incubated with and without SiO_2 (0.75 mg/ml Dörentrup Quartz DQ 12) at 37 °C overnight. The 20,000 × g supernatants of the sample were divided into small aliquots and stored at − 70 °C.

Granulation tissue was induced by s.c. implantation of viscose-cellulose sponge (Kongsfoss Fabrikker A/S, Oslo 2, Norway) into 3-month-old female albino rats[6]. Polysomes were prepared from 14-day experimental granulation tissue

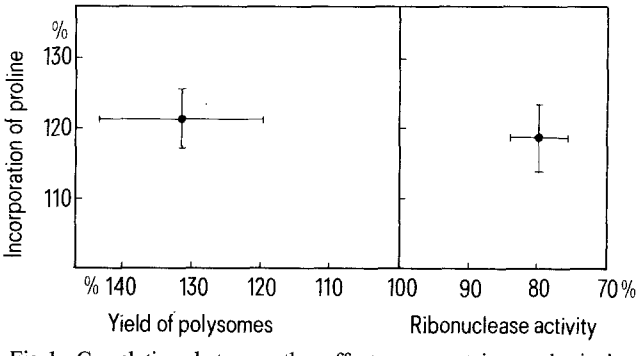

Fig. 1. Correlation between the effects on protein synthesis in granuloma slices and the polysome yield (left) and between the effects on protein synthesis and the RNase activity (right; granuloma polysomes as substrate) of silica-treated macrophage supernatant. The values are expressed in percent of the relevant control values with untreated macrophages (= 100%). The means ± SEM are given (n = 14).