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ISOLATION AND SYNTHESIS OF A NOVEL IMMUNOSUPPRESSIVE 17α-SUBSTITUTED DAMMARANE FROM THE FLOUR OF THE PALMYRAH PALM *(Borassus flabellifer)*

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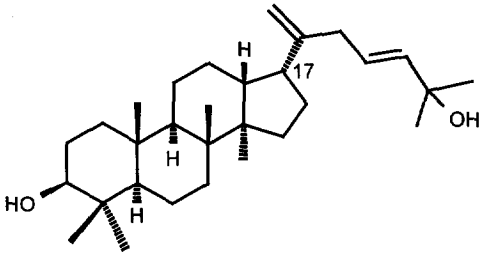
Abstract: The novel triterpene **1** with a dammarane skeleton and a hitherto unknown 17α-substitution pattern has been isolated from the Palmyrah palm in low yield and prepared by synthesis in larger quantities. **1** was shown to be an extremely potent immunosuppressant *in vitro* (MLR; IC_{50}=10 ng/ml) and *in vivo* (DTH; ED_{50}=0.01 mg/kg p.o.). A glucocorticoid like activity is excluded.

Introduction

The Palmyrah palm is widely distributed in tropical regions of the Asian continent. In Sri Lanka the outer portion of the young shoot is boiled, dried and milled to provide an edible flour. It has been reported[1-3] that the flour induces immunosuppression; in the northern province of Sri Lanka, where the flour is eaten extensively, the incidence of human malignant tumors is 3-4 times higher than in the rest of the country[4]. In the course of our continuing interest in the discovery of immunosuppressive agents, we isolated the novel triterpene **1** [(17α)-23-(E)-dammara-20,23-diene-3β,25-diol] from this flour and report here on its structure elucidation, synthesis and immunosuppressive properties.



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Isolation and structure elucidation

The isolation process of **1** was bioassayed by MLR (Murine Mixed Lymphocyte Reaction[5]) and consisted of extracting 5 x 10 kg of Palmyrah palm *(Borassus flabellifer)* flour with ethyl acetate, repartitioning

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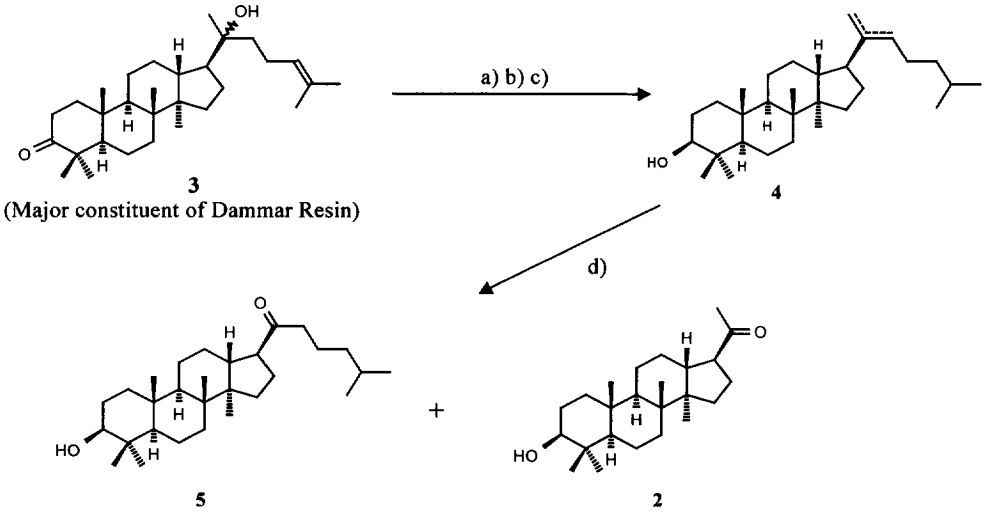
the evaporated extract between 90% aqueous methanol and hexane, followed by purifying the lower phase by several chromatographic steps[6]. The analysis of the resulting white amorphous powder obtained in low yield (0.5 mg / 50 kg) by ^{1}H-NMR, ^{13}C-NMR and FABHRMS revealed **1** to be a tetracyclic triterpene[7], which belonged to the class of dammaranes. The latter are found in many plants, e.g. the Ginseng roots and commonly bear a 17β–substituent. Dammaranes with an α-substituent in position 17 are new, **1** being the first representative. The structural proof for the 17α-configuration of the side chain in **1** is based on NOEs from a 2D ROESY spectrum, where a crucial NOE is observed between H-C(21) and H-C(30). ^{13}C-NMR shift differences between **1** and its 17β-epimer **11** are in agreement with the expected values[7,15] and finally, synthesis of **1** and **11** confirms the structural assignments.

Synthesis

The low yield of the isolation process and the potent immunosuppressive properties of **1** called for a synthesis, which was realized by employing the 17β-substituted dammarane **2**[8] as crucial intermediate. The synthesis of **2** (Figure 1) started from commercially available **3** (the major constituent of Dammar Resin, Fluka). In a first

Figure 1



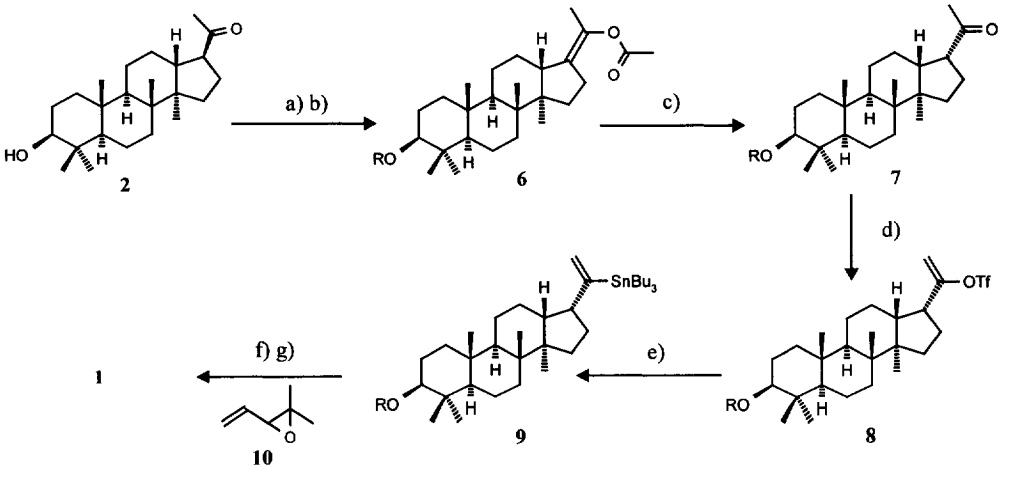
a) $NaBH_4$, iPrOH, r.t. 3.5h, 24%, based on weight of Dammar Resin. b) Pd/C, EtOH, H_2, 3h, 95%.
c) DMSO, 190° C, 3h, 38%. d) CH_2Cl_2, MeOH, O_3, Ph_3P, 50% for **2**; 12% for **5** after chromatography.

step, Dammar Resin was reduced by $NaBH_4$ to the equatorial 3-OH derivative of **3**. The double bond

between C-24 and C-25 was hydrogenated next in order to avoid complications in the ozonolysis to follow. The tertiary alcohol of the resulting saturated diol was dehydrated by heating in DMSO to deliver **4** as a mixture of olefins. This mixture underwent ozonolysis and rendered ketones **5** and **2** in a ratio of 4:1, which was separated by chromatography. The overall yield from Dammar Resin was 4.3 %.

The conversion of **2** into its 17α-epimer **7** required a three-step sequence: **2** was first protected as the 3-*tert.*butyl-diphenylsilyl ether **2a**, then refluxed with acetic anhydride to yield the enolacetate **6**, which upon reaction with MeLi and protonation with methyl salicylate generated the 17α-ketone **7** in respectable yield. The ratio of **7** and **2a** was 9:1; the α-ketone **7** could easily be separated from the β-ketone **2a** by silica gel chromatography. Vinyl triflate **8**[9] was obtained by deprotonating **7** with $KN(SiMe_3)_2$ followed by reacting with $PhNTf_2$[10]. Since direct coupling of vinyltriflate **8** and vinyl epoxide **10**[11] under $PdCl_2(CH_3CN)_2$ mediated catalysis gave no useful products, vinyltriflate **8** was converted into vinylstannane **9**[12] by reacting with $(Bu_3Sn)_2CuCNLi_2$[13]. The Stille[11] conditions were successfully applied for the coupling of vinylstannane **9** and vinyl epoxide **10** and delivered the desired target **1** after silyl ether cleavage (Figure 2).

Figure 2

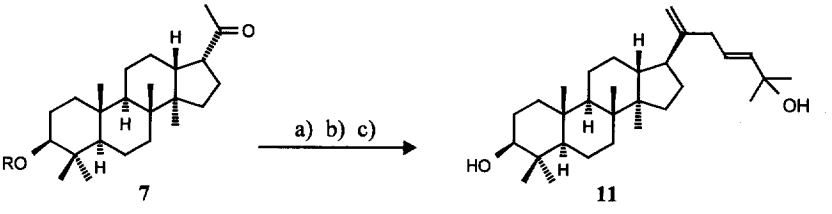


a) $tBuPh_2SiCl$, imidazole, CH_3CN, 60°C, 12h, 87% of **2a**. b) pTsOH, Ac_2O, rf, 7.5h, 72%. c) i: Et_2O, MeLi, 0°C, 30min. ii: -78°C, add methyl salicylate, 40min., 47%. d) $KN(SiMe_3)_2$, $PhNTf_2$, -78°C to 0°C, THF, 67%. e) $(Bu_3Sn)_2CuCNLi_2$, THF, -78°C, 63%. f) **10**, $Pd(CH_3CN)_2Cl_2$, DMF, THF, 12h, r.t., 90%. g) Bu_4NF, THF, 60°C, 1.5h, 92%. R: $tBuPh_2Si$-. rf: reflux. r.t.: room temperature.

The 17β-epimer **11** of **1** was readily prepared from α-ketone **7** (Figure 3) via the Shapiro reaction[14]: the triisopropylbenzenesulfonyl hydrazone derivative of **7** - which under the acidic reaction conditions of the

hydrazone formation underwent epimerization to the 17β–isomer - was deprotonated with n-BuLi to generate the vinyl lithium species and coupled under Cu-catalysis with vinyl epoxide **10** to provide **11**[15] in good yield.

Figure 3



a) 2,4,6-triisopropylbenzenesulfonyl hydrazide, CH_3CN, HCl_{aq}., r.t., 30min., 94%.
b) i: Et_2O, n-BuLi, -78°C, then -20°C. ii: -78°C, add CuCN, **10**, then -20°C, 2h.
c) THF, Bu_4NF, 50°C, 12h, 37% over 2 steps. R: $tBuPh_2Si$-

Biological Evaluation

In vitro and *in vivo* immunosuppressive activities of the 17α-substituted **1** were compared to the 17β–substituted **11** and Cyclosporin A (Table 1). In the serum-free MLR[5], **1** showed an IC_{50} of 10-40 ng/ml and was almost equipotent with Cyclosporin A, whereas the 17β-epimer **11** was significantly weaker. No cytotoxicities in the P-815 mastocytoma and the Jurkat cell line were observed[16]. **1** showed potent activity in the delayed type hypersensitivity (DTH) model induced by SRBC-T_H cells[17] in the mouse with an ED_{50}=0.01 mg/kg p.o. Cyclosporin A and the 17β-epimer **11** were considerably weaker in this assay. Pharmacokinetic studies demonstrated a good oral bioavailability of **1** with a half life of 5 h (data not shown). A glucocorticoid like activity could be excluded, since **1** was inactive in the BPNOD[18] -induced oedema in the rat. Chronic activity and the mechanism of action of this highly interesting new compound are currently under investigation.

Table 1

	MLR[a]; IC_{50} (ng/ml)	DTH[b]; ED_{50} (mg/kg p.o.)
1	10	0.04
11	800	10
Cyclosporin A	4	45

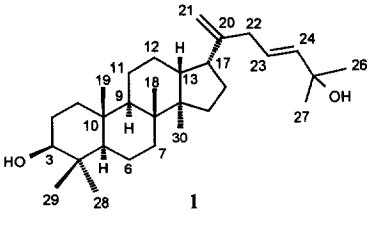
a) Serum-free Mixed Lymphocyte Reaction[5]
b) Delayed type hypersensitivity model in the mouse[17]
p.o.: oral administration

Acknowledgements

The authors express their appreciation to the Analytical Department of Novartis Pharma AG Basel for NMR & MS spectra including their interpretation. The Kilolab is greatfully acknowledged for the preparation of ample quantities of **2**.

References and Notes

1. Panabokke, R.G. Arseculeratne, S.N. Proc. Malignant lymphomas in rats after prolonged feeding with palmyrah (*Borassus flabellifer)* flour. *Sri Lanka Assoc. Adv. Sci.*, **1977**, *35*, 5. Arseculeratne, S.N. *Ceylon Med. J.* **1991**, *36*, 137.
2. Arseculeratne, S.N.; Sirisinka, S.; Charupatana, C.; Kangwangpong, D. Immunological alterations in rats fed with flour from palmyrah palm *(Borassus flabellifer). Proc. Soc. Exp. Biol. Med.* **1981**, *168*, 356.
3. Greis, J.B.; Kay, S.J.E.; Benetts, R.J. A toxin from the palmyrah palm, *Borassus flabellifer*: partial purification and effects in rats. *Food Cosmet. Toxicol.* **1980**, *18*, 483.
4. Kangwangpong, D.; Arseculeratne, S.N.; Sirisinka, S. Clastogenic effects of aqueous extracts of plamyrah *(Borassus flabellifer)* on human blood lymphocytes. *Mutat. Res.* **1981**, *89*, 63.
5. Meo, T. The MLR in the mouse. In: *"Immunological Methods"*, Lefkovits, L.; Pernis, B. Eds., Academic Press, N.Y. pp. 227-239.
6. Details of the isolation process will be published elsewhere
7. The spectra of isolated and synthetic **1** are identical. ^{1}H-NMR (360 MHz, $CDCl_3$): δ 0.73 (bd, 1H, J=12.5 and 1.3 Hz; H-C(5)); 0.78 (s, 3H, H-C(28)); 0.85 (s, 3H, H-C(19)); 0.90 (s, 3H, H-C(30)); 0.95 (s, 3H, H-C(18)); 0.98 (s, 3H, H-C(29)); 1.33 (s, 6H, H-C(26,27)); 1.19-1.74 (m, 15H, H-C(1, 2, 6, 7, 9, 11, 12, 15)); 1.75-1.84 (m, 2H, H-C(16)); 1.98-2.05 (m, 1H, H-C(13)); 2.59-2.64 (m, 1H, βH-C(17)); 2.66 (bd, 1H, H-C(22)); 2.79 (bs), 2.82 (dd, 1H, H-C(22)); 3.18-3.23 (m, 1H, αH-C(3)); 4.88 (s, 1H, H-C(21)); 4.95 (s, 1H, H-C(21)); 5.59-5.62 (m, 2H, H-C(23,24)).



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FABHRMS m/e = 443 (MH+).
^{13}C NMR [125.7 MHz, $CDCl_3$ (characteristic signals selected for comparison with **11**, sorted by C-atoms)]: δ 39.01 (C1); 27.38 (C2); 78.91 (C3); 38.96 (C4); 55.79 (C5); 18.28 (C6); 34.97 (C7); 40.78 (C8); 50.81 (C9); 37.11 (C10); 28.24 (C16); 43.78 (C17); 15.76 (C18); 16.22 (C19); 151.2 (C20); 110.27 (C21); 41.32 (C22); 125.44 (C23); 139.48 (C24); 70.7 (C25).
mp of **1** : 90.6-91.4°C (ether/hexane). $[\alpha]_{589}^{25}$ = -7.45. $[\alpha]_{296}^{25}$ = -109.5 (EtOH, c = 1.06).
HPLC retention time: 11.6min. (Merck LiChrospher 100 Rp-18 (5μm), 4x250mm; acetonitrile at 1.5ml/min.; detection at 210nm using a Waters 996 photodiode array detector)

8. Mills, J.S. *J.Chem.Soc.* **1956**, 2196. Mills, J.S.; Werner, A.E.A. *J.Chem.Soc.* **1955**, 3132. Labriola, R.; Ourisson, G. *Tetrahedron* **1973**, *29*, 2105. Tori, M.; Tsuyuki, T.; Takahashi, T. *Bull.Chem.Soc.Jpn.* **1977**, 3349.
9. Vinyltriflate **8** was obtained as a crystalline compound after chromatography on silica gel (ether/hexane 3:97). ^{1}H.NMR (360 MHz, $CDCl_3$), characteristic signals: δ 2.85-2.94 (bq, 1H, βH-C(17); 3.20 (dd, 1H, αH-C(3)); 5.12 (bd, 1H, J=4Hz, H_a-C(21); 5.20 (d, 1H, J=4Hz, H_b-C(21).
10. Ireland, R.E.; Thaisrivongs, S.; Dussault, P.H. *J.Am.Chem.Soc.* **1988**, *110*, 5768.
11. Tueting, D.R.; Echavarren, A.M.; Stille, J.K. *Tetrahedron* **1989**, *45*, 979.
12. Vinylstannane **9** was obtained as a colorless viscous oil after chromatography on silica gel (hexane). ^{1}H.NMR (360 MHz, $CDCl_3$), characteristic signals: δ 2.82-2.92 (bq, 1H, βH-C(17); 3.14 (dd, 1H, αH-C(3)); 5.18 (bs, 1H; H_a-C(21); 5.80 (bs, 1H, H_b-C(21).
13. Gilbertson, S.R.; Challener, C.A.; Bos, M.E.; Wulff, W.D. *Tetrahedron Lett.* **1988**, *29*, 4795.
14. Shapiro, R.H. *Org.React. (N.Y.)* **1976**, *23*, 405. Chamberlin, A.R.; Bloom, S.H. *ibid.* **1991**, *39*, 1.
15. De Pascual Teresa J., Bellido, I.S.; Gonzalez, M.S.; Vicente, S. *Phytochemistry* **1986**, *25*, 185.
^{1}H.NMR (360 MHz, $CDCl_3$), characteristic signals: δ 2.12-2.18 (1H, m, αH-C(17)); 2.55-2.65 (2H, m, H-C(22)); 3.13 (1H, dd, J=5 and 12 Hz, αH-C(3)); 4.62 (1H, s, H-C(21); 4.70 (1H, s, H-C(21)); 5.50-5.60 (2H, m, H-C(23,24)).
^{13}C NMR [125.7 MHz, $CDCl_3$ (characteristic signals selected for comparison with **1**, sorted by C-atoms)]: δ 38.98 (C1); 27.41 (C2); 78.93 (C3); 39.14 (C4); 55.93 (C5); 18.30 (C6); 35.45 (C7); 40.50 (C8); 50.95 (C9); 37.12 (C10); 28.86 (C16); 45.32 (C17); 15.81 (C18); 15.66 (C19); 151.4 (C20); 108.2 (C21); 37.24 (C22); 125.04 (C23); 139.43 (C24); 70.0 (C25).
16. Staehelin, H. *Med.Exp.* **1962**, *7*, 92.
17. Bianchi, A.T.J.; Hoojikaas, H.; Brenner, R.; Tees, R.; Nordin, AA.; Schreier, M.H. *Nature* **1981**, *290*, 62. Herrmann, P.; Schreier, M.H.; Borel, J.-F.; Feuer, C. *Int. Arch. Allergy appl. Immunol.* **1988**, *86*, 102.
18. BPNOD: Bis-Pyridyl-N-Oxid-Disulfide.