Phytochemistry, Vol. 24, No. 9, pp. 2051–2054, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

SEPARATION OF THE PENTACYCLIC TRITERPENES TYLOLUPENOLS A AND B FROM *TYLOPHORA KERRII*

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(*Revised received* 2 *January* 1985)

Key Word Index—*Tylophora kerrii*; Asclepiadaceae; tylolupenols A and B.

Abstract—Tylolupenols A and B from *Tylophora kerrii* were separated and identified as D:C-friedolup-8(9)-en-3β-ol and D:C-friedolup-9(11)-en-3β-ol, respectively.

INTRODUCTION

Tylolupenols A and B were isolated as a mixture from the root of *Tylophora kerrii* Craib. (ren shen wa er teng in Chinese) collected in Yun nan Lit Cang, China, and their chemical structures were elucidated as D:C-friedolup-8(9)-en-3β-ol and D:C-friedolup-9(11)-en-3β-ol, respectively, by an X-ray study of a 1:1 molecular complex of their acetates [1, 2]. In this paper, their separation is described as well as their physical and spectroscopic properties and some chemical reactions.

RESULTS AND DISCUSSION

Tylolupenols A and B, as well as their acetates, had been considered as one compound, since only one spot appeared on thin layer chromatograms, until ^{13}C NMR and X-ray analysis showed them to be different compounds [1, 2]. Several solvent systems were tested for their separation on TLC and HPLC. Two compounds were separated on HPLC (silica gel) by *n*-hexane saturated with water–*i*-propylether–*i*-propanol (97.5:2.00:0.25). However, no other solvent system separated them completely. We have used an automatic recycling HPLC with the solvent described above to separate them preparatively.

Tylolupenol A (**1**, $C_{30}H_{50}O$), mp 184–185°, recrystallized from methanol, $[\alpha]_D$ + 34.3° ($CHCl_3$), gave a reddish brown colour by the Liebermann–Burchard test. The presence of hydroxy (ν3620 cm^{-1}) and alkane (2950, 2870, 1460 and 1380 cm^{-1}) groups was shown by the IR spectrum. Protons for six singlet angular methyl groups, two doublet methyl groups of an isopropyl group, and a multiplet methine group bearing a hydroxy group were detected in the ^{1}H NMR spectrum (Table 1). INEPT (insensitive nuclei enhanced by polarization transfer) on ^{13}C NMR refocussed methyl, methylene and methine carbons revealed eight methyl carbons, a methine carbon (δ79.01) bearing a hydroxy group and two quaternary carbons (δ133.92 and 134.58) on a double bond in the ring (Table 2). The remaining carbons detected by the INEPT spectrum suggested that tylolupenol A was a pentacyclic triterpene alcohol having a tetrasubstituted double bond and a five-membered ring E to which an isopropyl group was attached. The mass spectrum showed prominent peaks for a pentacyclic triterpene alcohol having a double bond at Δ^8 or $\Delta^{9(11)}$ (Table 3) [3].

Table 1. ^{1}H NMR spectra of tylolupenols A and B and their derivatives

	Angular methyl	*i*-Propyl methyl	Methine on double bond	Methine bearing hydroxy	Acetyl methyl
Tylolupenol A (**1**)	0.81 (2)*, 0.94, 0.97, 0.98, 1.00	0.92 (*d*, *J* = 7.0 Hz) 0.96 (*d*, *J* = 7.0 Hz)		3.24 (*m*)	
Tylolupenyl A acetate (**2**)	0.80, 0.87 (3), 0.97, 0.98	0.92 (*d*, *J* = 7.0 Hz) 0.95 (*d*, *J* = 7.0 Hz)		4.51 (*dd*, *J* = 11.0, 5.5 Hz)	2.05
Tylolupenol B (**3**)	0.68, 0.77, 0.88, 0.92, 0.96, 1.07	0.88 (*d*, *J* = 7.0 Hz) 0.89 (*d*, *J* = 7.0 Hz)	5.35 (*m*)	3.22 (*m*)	
Tylolupenyl B acetate (**4**)	0.68, 0.76, 0.84, 0.92, 0.94, 1.09	0.87 (*d*, *J* = 6.0 Hz) 0.90 (*d*, *J* = 6.0 Hz)	5.35 (*m*)	4.49 (*dd*, *J* = 9.5, 7.0 Hz)	2.06
Tylolupenone (**5**)	0.72, 0.76, 0.93, 1.05, 1.14, 1.32	0.89 (*d*, *J* = 6.0 Hz) 0.90 (*d*, *J* = 6.0 Hz)	5.43 (*m*)		

*(2) = two methyl, (3) three methyl, otherwise one methyl.

Table 2. ^{13}C NMR spectra of tylolupenols A and B and their derivatives

	Tylolupenol A (1)	Tylolupenyl A acetate (2)	Tylolupenol B (3)	Tylolupenol B acetate (4)	Tylolupenone (5)	Neolup-13(18)-en-3β-yl (6)
CH_3	15.59	16.67	14.39	14.39	14.42	18.34
	16.85	16.78	15.11	15.99	16.04	21.77
	19.91	19.97	16.07	16.19	21.68	23.70
	21.20	21.20	22.36	21.33	22.34	25.77
	21.96	21.96	23.38	22.34	23.36	27.83
	23.37	22.33	25.37	23.36	24.34(2)*	34.64
	28.04	23.37	27.48	25.37	32.57	35.59
	33.16	28.00	32.58	27.42		38.62
		33.16		32.58		41.58
CH_2	19.25	19.09	18.33	18.23	18.21	16.48
	20.79	20.79	19.28	19.09	19.42	16.59
	25.18	24.22	27.61	24.67	27.62	17.56
	27.73	25.21	28.19	27.60	28.97	20.12
	27.94	27.58	28.99	28.97	32.25	21.18
	28.49	28.49	32.30	32.28	35.16	21.31
	31.05	31.02	37.65	37.61	37.60	21.93
	32.69	32.67	38.33	38.31	38.29	25.17
	34.99	34.69	39.27	38.91	40.42	25.40
	40.84	40.81				28.03
CH	36.35	36.35	35.88	35.88	35.88	33.71
	48.91	48.91	40.95	40.89	40.75	47.70
	50.58	50.67	44.24	44.42	46.35	50.84
	54.08	54.08	49.44	49.44	49.48	55.58
	79.01	80.96	53.98	53.98	53.98	80.96
			79.17	81.03	116.91	
			115.96	116.20		
C	36.89	36.90	36.76	36.74	36.83	37.28
	37.68	37.52	36.95	36.97	37.00	37.82
	38.82	37.73	37.74	38.11	40.75	40.80
	40.55	40.55 (2)	41.34 (2)	41.34 (2)	41.31	41.15
	40.95	134.03	150.20	149.91	48.07	44.90
	133.92	134.44		171.02	148.65	133.38
	134.58	171.02			216.83	141.13
						171.00

*(2) = Two carbons.

Tylolupenol A acetate (**2**, $C_{32}H_{52}O_2$) was identified by IR (carbonyl at ν1720 cm^{-1}), ^1H NMR (Table 1) and ^{13}C NMR (Table 2). The mass spectrum showed a similar fragmentation pattern to the original alcohol (Table 3). A ^1H NMR coupling constant (J = 11.0 and 5.5 Hz) of the methine proton bearing an acetyl group at δ4.51 (Table 1) suggested the acetyl group to be in the β-orientation at C-3. Thus, tylolupenol A was assigned as D:C-friedolup-8(9)-en-3β-ol (**1**).

Tylolupenol B (**3**, $C_{30}H_{50}O$), mp 177–178° recrystallized from methanol, $[\alpha]_D$ −6.1°, gave a reddish brown colour by the Liebermann–Buchard test. Hydroxy and alkane groups were detected at 3620 and 2950, 2870, 1460 and 1380 cm^{-1} in the IR spectrum. Six angular methyl groups, an isopropyl group and two methine carbons bearing a hydroxy group and a double bond, respectively, were identified from the ^1H NMR spectrum (Table 1). These groups were also observed from the ^{13}C NMR spectrum (Table 2). The remaining carbons assigned by INEPT (Table 2) revealed tylolupenol B to be a pentacyclic triterpene alcohol having a trisubstituted double bond and a five membered ring E to which an isopropyl group was attached. The mass spectrum of tylolupenol B was similar to that of tylolupenol A (Table 3).

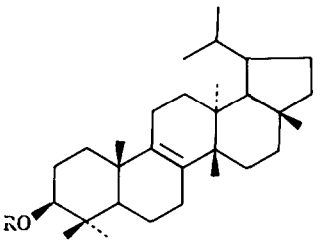
Tylolupenol B acetate (**4**, $C_{32}H_{52}O_2$) was prepared and identified by its IR, ^1H NMR, ^{13}C NMR and mass spectra. A ^1H NMR coupling constant (J = 9.5 and 7.0 Hz) for the methine proton bearing an acetyl group showed that it was substituted in the β-orientation at C-3. Thus, tylolupenol B was identified as D:C-friedolup-9(11)-en-3β-ol (**3**).

Oxidation of a mixture of tylolupenols A and B with Jones reagent gave only one product (**5**, $C_{30}H_{48}O$) mp 212–213° [4]. It showed a carbonyl group absorption at 1705 cm^{-1} and no hydroxy group was detected in the IR spectrum. Six angular methyl groups, an isopropyl group and a methine group on a double bond were apparent from the ^1H NMR spectrum (Table 1). Eight carbons for methyl groups, two carbons for a trisubstituted double bond and a carbonyl carbon were seen in the ^{13}C NMR spectrum (Table 2). The mass spectrum is shown in Table 3. These data suggested that the oxidation product (**5**) was the oxide of only tylolupenol B (**3**). Therefore, it is concluded that the Δ^8-bond of tylolupenol A (**1**) has

Table 3. Mass spectra of tylolupenols A and B and their derivatives

	$[M]^+$	Fragments			
Tylolupenol A (1)	426 (45.8)*	412 (31.1) ($C_{29}H_{48}O$)	411 (97.3) ($C_{29}H_{47}O$)	259 (100.0) ($C_{18}H_{27}O$)	241 (39.4) ($C_{18}H_{25}$)
	($C_{30}H_{50}O$)	229 (19.5) ($C_{17}H_{25}$)	206 (26.4) ($C_{15}H_{26}$)	205 (36.8) ($C_{15}H_{25}$)	163 (11.3) ($C_{12}H_{19}$)
		149 (11.1) ($C_{11}H_{17}$)	123 (33.4) (C_9H_{15})		
Tylolupenyl A	468 (12.6)	453 (31.2) ($C_{31}H_{49}O_2$)	301 (35.0) ($C_{20}H_{29}O_2$)	241 (27.6)	229 (14.2)
acetate (2)	($C_{32}H_{52}O_2$)	205 (29.2)	123 (42.6)	43 (100.0)	
Tylolupenol B (3)	426 (27.9)	412 (21.5)	411 (66.8)	259 (67.4)	241 (28.1)
		229 (15.5)	206 (28.9)	205 (28.9)	189 (12.5) ($C_{14}H_{21}$)
		163 (20.0)	149 (14.2)	123 (81.5)	
Tylolupenyl B	468 (11.8)	453 (31.2)	393 (9.6) ($C_{29}H_{45}$)	301 (28.4)	241 (19.9)
acetate (4)		229 (9.0)	205 (27.3)	123 (61.8)	43 (100.0)
Tylolupenone (5)	424 (24.5)	409 (40.6) ($C_{29}H_{45}O$)	257 (100.0) ($C_{18}H_{25}O$)	245 (24.5) ($C_{17}H_{25}O$)	205 (80.8)
	($C_{30}H_{48}O$)	123 (87.9)			

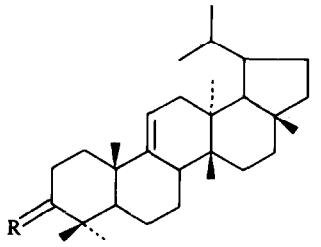
*m/z (rel. int.).



R

1 H

2 Ac

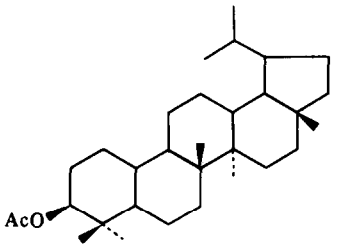


R

3 H,OH(β)

4 H, OAc(β)

5 O



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migrated to the $\Delta^{9(11)}$ position during Jones oxidation.

A mixture of tylolupenol acetates was treated with hydrobromic acid–acetic anhydride in chloroform–phenol to obtain neolup-13(18)-en-3β-yl acetate (**6**) [lup-13(18)-en-3-ol acetate (3β,19β)], for which the ^{1}H NMR spectrum was identical with one of the corresponding specimens derived from neolupenyl acetate [5]. In this reaction the double bonds at Δ^8 of tylolupenol A and $\Delta^{9(11)}$ of tylolupenol B migrated to the $\Delta^{13(18)}$ position with a sequential shift of the methyl groups. Therefore, it was concluded that the isopropyl group and a methyl group on E ring of the tylolupenols had the 19β- and 22β-orientations, respectively.

The structures of tylolupenols A and B were therefore proved to be D:C-friedolup-8(9)-en-3β-ol (**1**) and D:C-friedolup-9(11)-en-3β-ol (**3**), respectively.

EXPERIMENTAL

Separation of tylolupenols A and B. The mixture of tylolupenols A and B was chromatographed on silica gel (μ porasil) column (4.6 × 300 mm) in a Waters Associate Liquid Chromatograph, Model 441. Absorbance detector (214 nm), using *n*-hexane saturated with H_2O–*i*-Pr_2O–*i*-PrOH (97.75:2.00:0.25), flow rate 1.0 ml/min. Eight automatic recyclings separated tylolupenol A from tylolupenol B.

Tylolupenol A (**1**). $C_{30}H_{50}O$ (426.384 for 426.386), mp 184–185°, $[\alpha]_D$ +34.3° ($CHCl_3$); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 239 (2.489); CIMS (*i*-butane) *m/z*: 427 $[M+1]^+$. *Tylolupenol A acetate* (**2**). $C_{32}H_{52}O_2$ (468.396 for 468.396), CIMS (*i*-butane) *m/z*: 468.

Tylolupenol B (**3**). $C_{30}H_{50}O$ (426.390 for 426.386), mp 177–178°, CIMS (*i*-butane) *m/z*: 427 $[M+1]^+$. *Tylolupenol B acetate* (**4**). $C_{32}H_{52}O_2$ (468.402 for 468.396), CIMS (*i*-butane) *m/z*: 468.

Oxidation of a mixture of tylolupenols A and B. The mixture (20 mg) in Me_2CO (40 ml) was oxidized at room temp. with Jones reagent added dropwise until a persistent orange colour was obtained. The ppt from the reaction mixture was filtered off and washed with Me_2CO. All filtrates were combined and chromatographed on a silica gel column using 1% *i*-Pr_2O in *n*-hexane to obtain tylolupenone (**5**), $C_{30}H_{48}O$ (424.370 for 424.370); mp 212–213°; $[\alpha]_D$ −14.0° ($CHCl_3$); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 240 (2.522), 252 sh (2.337); CIMS (*i*-butane) *m/z*: 425 $[M+1]^+$.

Migration of double bonds. Tylolupenol acetates A and B (5 mg) were dissolved in $CHCl_3$ (1 ml) and phenol (0.5 ml) into which Ac_2O (0.6 ml) and HBr (47%) (0.15 ml) were added. After refluxing for 3 hr, $CHCl_3$ was removed and the residue was poured into ice. The precipitate was filtered off and crystallized from Me_2CO. $C_{32}H_{52}O_2$ (468.397 for 468.396), mp 182–183°, CIMS (*i*-butane) *m/z*: 468.

Acknowledgements—We are grateful to Drs. M. Sugiura and K. Saiki of our school for NMR and mass spectra, respectively.

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