KOMBIC ACID, A HYDROQUINONE POLYISOPRENOIC CARBOXYLIC ACID FROM PYCNANTHUS KOMBO SEED FAT

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Key Word Index—Pycnanthus kombo; Myristicaceae; seed fat; kombic acid; hydroquinone polyisoprenoic carboxylic acid.

Abstract—The fat of the seeds from the West African tree *Pycnanthus kombo* contains ca 20% of a hitherto undescribed compound. This compound was identified as 16(2',5'-dihydroxy-3'-methylphenyl)-2,6,10,14-tetramethyl-2,6,10,14-hexadecatetraenoic acid, for which the name kombic acid is proposed.

INTRODUCTION

The seeds of the West African tree *Pycnanthus kombo* [1, 2] belonging to the Myristicaceae, contain, on average, *ca* 55% of a dark brown fat which is characterized by the presence of relatively high contents of tetradecanoic (60%) and (*Z*)-9-tetradecenoic acid (20%) [3, 4]. Atherton and Meara [3] also noted the occurrence in the fat of what they surmised to be highly unsaturated resin acids.

Our examination of the *P. kombo* fat showed the presence of a unique, hitherto undescribed compound constituting ca 23 % by wt of the total fat extracted. This compound was isolated directly by chromatography of the whole fat and identified as 16(2',5'-dihydroxy-3'-methylphenyl)-2,6,10,14-tetramethyl-2, 6,10,14-hexadeca-tetraenoic acid, for which we would propose the name kombic acid (1).



RESULTS AND DISCUSSION

Kombic acid (1) could be converted into its methyl ester (2) by treatment with diazomethane. A diacetate (3) was formed on treatment of 2 with acetic anhydride and a dimethyl ether (4) by alkylation with methyl iodide. These

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conversions showed the presence of a carboxyl group and two hydroxy functions. The mass spectrum of 2 showed its molecular formula to be $C_{28}H_{40}O_4$.

Comparison of the UV absorption maximum of kombic acid at 290 nm (in methanol), and its shift to 304 nm on addition of base (sodium hydride), with the spectral data of methyl hydroquinone and 4- and 5methylresorcinol [5] suggested a hydroquinone structure. This was confirmed by oxidation of 2 with activated manganese dioxide, yielding the substituted paraquinone (5). An orthoquinone structure could be excluded by the lack of an absorption band at 550-600 nm [6]. The presence of a shoulder at ca 450 nm ($\varepsilon = 20$) in ethanol is also in agreement with a paraquinone structure, which usually exhibits a low intensity maximum at 430-460 nm [6]. The IR spectrum of the oxidation product also contained absorptions characteristic of paraquinones. The quinone (5) was readily converted to 2,8-dimethyl-2-(4,8-dimethyl-12-methoxycarbonyl-3, 7, 11-tridecenyl)-2H-chromen-6-ol (7) on heating in pyridine.

¹H NMR data of compounds 1, 3, 4 and 6 are presented in Table 1. The data of kombic acid (1) indicated the presence of four isoprene units. Besides the four vinylic methyl groups of the polyisoprene chain, the signal at $\delta 2.17$ indicates that a fifth methyl group is attached to an aromatic ring. The doublet at $\delta 3.29$ is indicative of benzylic hydrogens of isoprenoid benzene derivatives. The chemical shifts and intensities of the signals at δ 6.48 and 6.51 indicate the presence of two non-equivalent aromatic protons. The magnitude of the coupling constant (J = 2.6 Hz) shows that these aromatic protons are meta-coupled. Additional evidence for the given substitution pattern of the aromatic ring stems from the observed paramagnetic shift, induced by the shift reagent Eu(fod)₃, in the ¹H NMR spectrum of 3. The signals at δ 6.77 and 6.82 show an identical shift to lower field upon addition of the shift reagent, which indicates that the aromatic protons are equidistantly situated with regard to the acetate groups. The presence of a 2-substituted-2unsaturated carboxylic acid was indicated by a triplet at $\delta 6.02$ and a quartet at 2.59. Irradiation of the $\delta 2.59$ quartet caused the triplet at 6.02 to collapse into a singlet. Reduction of the dimethyl ether (4) with lithium alumi-

				OR1											
		(3) (2)	<u>(</u>)	J J J	-	(a) Me	i.	(a) Me	Ē	(a) Me	Э. Н — С С — Н — С	e)			
			Ŭ.	רד סג _ו (ה	13 – CH = (i)	(q) 	(h) - CH	<u>ب</u> ا	П ₂)2 — СF () (g.)	$1 = U - UH_2 - UH_2$) (d) (e)		~			
	B	q	c	q	υ	ų	g,h			k,l	E	u	0	d	q,r
$\mathbf{I} \mathbf{R}_1 = \mathbf{H}$	1.58 s 1.67 s	1.9–2.2 c	2.17 <i>s</i>	2.27 t $J = 7.3$	2.59 q $J = 7.5$	3.29d $J = 7.0$	5.11 br t 5.14 br t	5.29t $J = 7.3$	6.02 t $J = 7.3$	$6.48 d \ 6.51 d$ $J = 2.6 J = 2.6$					
$R_2 = C_{OH}$	1.74 s														
3	1.58 s	1.9–2.2 c	2.14 <i>s</i>	2.25 br t	2.50 q	3.19 <i>d</i>	5.09 t	5.22 t	5.851	6.77 d 6.82 d			2.26 <i>s</i>	3.73 <i>s</i>	
$R_1 = C^2 Me(o)$	1.60 s				J = 7.5	J = 7.0	5.14 <i>t</i>	J = 7.3	J = 7.3	J = 2.5 J = 2.5			2.31 <i>s</i>		
	1.67 s						J = 7.3								
$R_2 = C_0 OMe(p)$	1.68 s														
4 $R_1 = Me(q)$	1.56 <i>s</i> 1.58 <i>s</i>	1.8–2.2 c	2.28 s	2.25 br t	2.50 <i>q</i> J = 7.5	3.34d $J = 7.3$	5.09 br t 5.15 br t	5.30 br t	5.86 <i>t</i> J = 7.3	6.56 <i>s</i>					3.67 s 3.72 s
$R_2 = C_{OMP(r)}^{0}$	1.66 <i>s</i>														3.73 s
	1.71 s														
$6 R_1 = Me(m)$ $R_2 = CH_2OH(n)$	1.60 <i>s</i> 1.69 <i>s</i> 1.73 <i>s</i>	1.9–2.2 c	2.28 s	1.9-2.2 <i>c</i>		3.36d $J = 7.3$	5.15 br t	5.32 t $J = 7.0$	5.32 t $J = 7.0$	6.60 s	3.70 <i>s</i> 3.76 <i>s</i>	4.12 <i>s</i>			
Solutions in CDCl ₃ eith	er at 300 (3)) or 200 MH	z (1, 4, 6)). <i>ð</i> -Values	auoted in	nop maa	ufield from	TMS are	accurate to	o within + 0.01 nnm	Comline	constants	are accura	te to withi	n + 0.2 Hz

Table 1. ¹H NMR data of kombic acid and derivatives

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Table 2. ¹H NMR data of compound 7

a	b	с	d,e	f	g	h	i	j,k	1	m	n	o,p
1.35 s	1.56 s (2 Me-gr .) 1.66 s (2 Me-gr .)	1.66 t J ~ 8	1.9–2.2c	2.12 s	2.24 t $J = 7.5$	2.50 q $J = 7.5$	3.73 s	5.09 t 5.14 t J = 7.0	5.56 d $J = 7.0$	5.85 t $J = 7.5$	6.24 d $J = 9.8$	6.33 d 6.48 d J = 2.6

nium hydride confirmed this assignment. The olefinic triplet at $\delta 6.02$ shifted to 5.32 and the multiplet at 2.59 then coincided with the other CH₂ signals present.

The geometric configuration of the 2-double bond could be established by the highly characteristic signals of 2-substituted-2-unsaturated acids [7, 8]. Comparison of the signal for 1 at $\delta 6.02$ with the olefinic signals for methyl esters of tiglic and angelic acids, which absorb at 6.97 and 6.18, respectively, shows the configuration of the first double bond of kombic acid to be that associated with angelic acid.

Kombic acid belongs to a class of natural terpenoids consisting of a polyisoprene system joined to a resorcinol, hydroquinone or quinone nucleus. In this class, only very few compounds occur with oxygen in the end-group of the terpenoid chain. Among the few examples known are alliodorin (8) from the bark of *Cordia alliodora* [9, 10] and pirolagenin (9) from *Pyrola japonica* [11]. Compounds with a carboxyl end-group have not been found in this class of terpenoids. In addition, kombic acid differs from other isoprenoid hydroquinones by the length of the side chain and in the substitution pattern of the aromatic ring. The configuration of the α -double bond is similar to that of 9 and opposite to that of 8.







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EXPERIMENTAL

P. kombo seeds (5 kg) were ground in a mill and extracted with petrol. Filtration and evaporation gave *ca* 2.8 kg fat, (56 % by wt) which had a dark coffee colour. The fatty acid composition (after saponification and re-esterification) was: 12:0 (5.6 %), 14:0 (64.4 %), 14:1 (19.4 %), 16:0 (2.2 %), 16:1 (2.6 %), higher than 18:0 (6.0 %) by GC. Kombic acid polymerized during this procedure. After isolation by distillation and urea fractionation, the 16:1 fraction was shown by ozonolysis, and by hydrogenation to Me 16:0, to consist of the Me Z-9- and Z-11-hexadecenoates in the ratio 55:45. The fat (1 kg), dissolved in a mixture of petrol and Et₂O, was applied to a column of Si gel (4 kg). The column was eluted with gradually increasing amounts of Et₂O in petrol until all the triacylglycerol material (680g) had been eluted at a petrol–Et₂O ratio of 4:1. Kombic acid (1) was finally eluted with Et₂O and obtained as a reddish-brown oil (230 g).

Alternatively, fat samples were treated with $Et_2O-CH_2N_2$ and the Me ester (2) was isolated in the same way. Yields were less (18-22% by wt based on fat), possibly due to Me ether formation and loss of more easily eluted material. TLC (Si gel; toluene- Et_2O , 4:1) indicated that, despite the brown colour, both products were essentially pure. Kombic acid is present in the fat in the free state; acid numbers for different samples of the brown fat were in the range 50-80.

Derivatives of kombic acid. The di Me ether (4) was prepared by dissolving 8.8 g Me ester () in 50 ml Me₂CO, and adding a soln of 2.5 g KOH in 5 ml H₂O to the ice-cold soln. The colour changed at once to a deep jade green, and MeI (10 ml) was added immediately with swirling. Within 10 min, TLC indicated complete conversion to 4 and the colour had reverted to redbrown. After CC (Si gel), pure 4 was obtained as a pale-yellow oil.

The other derivatives were prepared in mg to g amounts as follows. The diacetate, 3, from 2 by using excess Ac_2O and NaOAc as catalyst; the quinone, 5, from 2 with activated MnO_2 in petrol; the primary alcohol, 6, from 4 by reduction with LiAlH₄ in Et₂O. When the quinone, 5, was heated in pyridine, 7 was obtained. This compound was purified by chromatography and obtained in 65% yield as a pale-yellow oil. MS: parent peak m/z 438.

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