The Chemistry of Kitols

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The structure of kitol, a natural retinol dimer, was reinvestigated. Kitol-A and -B were isolated from whale liver and the former was recognized as "kitol" reported so far. Extensive ^1H -NMR (200 MHz) data for kitol-A and its related compounds (retinyl acetate-tetracyanoethylene adduct, -fumaronitrile adduct, and -crotononitrile adduct) were presented and the structure of kitol-A including its stereochemistry was determined decisively. Kitol-B is believed to be the mono-*cis* isomer of kitol-A.

Since the discovery of kitol, a natural retinol dimer initially found in whale liver oil,¹⁾ a large number of papers on its chemical structure have been published,²⁾ i.e. structures (I),^{3,4)} (II)⁵⁾ and (III)^{6,7)} were successively proposed for this natural product, while (IV)⁴⁾ for a pyrolysis product pseudokitol (Fig. 1). Kitol has since been regarded as a 1,4-cycloaddition product of the 11, 13-diene part of the first with the 13-monoene part of the second retinol molecule, leading to the formation of the central cyclohexene ring. Until recently, based mainly on 60 MHz ^1H -NMR data⁶⁾ or on mass spectral evidence,⁷⁾ the structure of the parallel addition type III has been generally accepted as the most favorable chemical structure of kitol, while the photodimerization of retinyl esters⁸⁻¹¹⁾ and chemical formation of kitol from retinol^{12,13)} have also been investigated in detail. In 1976, however, YANO¹⁴⁾ proposed a novel cyclopentane structure (V) and claimed an invalidity of the formula III described above. This statement prompted us to report our findings on the chemical structure and stereochemistry of kitol.¹⁵⁾ We now give here a full account of our results on the chemistry of kitol-A and -B together with their related compounds such as the tetracyanoethylene adduct (VI),¹⁶⁾ the fumaronitrile adduct (VII) and the crotononitrile adduct (VIII) with retinyl acetate (Fig. 1).

Crude kitol, isolated from whale liver, was successfully divided into two components, designated kitol-A (Rf 0.51) and kitol-B (Rf 0.45), by employing preparative thin-layer chromatography (pTLC) on a silica-gel plate. Although both kitol-A and -B have similar spectral characteristics, all experimental results demonstrated that the hitherto

known kitol was in excellent agreement with kitol-A but not with kitol-B.

It is obvious that the most important information for distinguishing the structure III from V can be obtained directly from ^1H - or ^{13}C -NMR data, e.g. the existence of the particular proton or carbon such as 11-H or 11-C in III and 14-H in V, number of protons attached to the carbinol carbons, or splitting pattern(s) of carbinol carbons in off-resonance measurement would be the point for this purpose. A number of NMR measurements employing various techniques were therefore undertaken. Of these, ^1H -NMR of kitol-A was completely resolved for the first time by the present authors, though assignment of some ^{13}C -NMR signals remained uninterpreted. Careful inspection of the 200 MHz ^1H -NMR data (Tables 1 and 2; Fig. 2) has eventually led us to the first recognition of the existence of the proton attached to a double allylic carbon atom, viz. 11-H in VI, VII, VIII, and then in III. Thus, it is now evident that the formula III is correct and V should be abandoned.

For determining the stereochemistry of kitol, BURGER *et al.*²⁾ attributed two one-proton-doublets at δ 5.42 (J 6 Hz) and 5.14 ppm (J 10.5 Hz) to 10-H and 12-H, respectively (*cf.* Table 2). Based on this interpretation they suggested that the trienyl chain attached to the 11-C position of the central ring would be in the quasi-axial conformation. However, from extensive decoupling experiments on kitol-A and its related compounds, especially on VII, we conclude that Burger's assignment on 10-H and 12-H as well as stereochemical interpretation around 11-C should now be revised as follows. We ascribe the one-proton-doublet at

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Fig. 1. The structures of kitol and model compounds.

δ 5.15 ppm to 10-H and the broad one-proton-singlet centered at 5.36 ppm (see Footnote *4 in Table 2) to 12-H. Moreover, $J_{10,11}$ and $J_{11,12}$ values are newly estimated as 9 and *ca.* 0 Hz, respectively, instead of 6 and 10.5 Hz as reported earlier. It would therefore be apparent that the above mentioned trienyl side chain orients in the quasi-equatorial direction at 11-C on a twist half-chair form of the cyclohexene ring. It was confirmed that kitol-A has similar NMR spectral characteristics as model compounds (VI-VIII), amongst which the stereochemistry of the adduct VII was determined decisively as (VIIa). Consequently, with acceptance of the validity of the stereochemical conclusion for the adducts and based not only on the spectral but also on the

chemical information accumulated so far,²⁾ the configuration (IIIa) (trienyl and tetraenyl chains are *trans* [equatorial and quasi-equatorial] and two hydroxymethyl groups are *cis* [equatorial and quasi-axial]) would be expected for kitol-A. This deduction involves further argument¹¹⁾ that the orbital overlap would proceed dominantly at 14-14' rather than at 12-12' in a concerted cycloaddition reaction of the first to the second retinol molecule.

Finally, kitol-B, the second component of a crude kitol isolated from whale liver, was investigated. At the present, it seems reasonable to determine kitol-B as the 9'-monocis isomer of kitol-A (all-*trans* form) from the following spectral features: λ_{max} in ethanol, 286 and 230 (infl.) nm;

Fig. 2. ^1H -NMR in CDCl_3 for kitol (III) and the retinyl acetate-fumaronitrile adduct (VII). (200 MHz; δ ppm from internal tetramethylsilane; resonances in a higher magnetic field were omitted).

Table 1. ^1H -NMR chemical shifts in CDCl_3 (δ ppm from internal tetramethylsilane) for the model compounds (VI)–(VIII)

Compound	MHz	CH_3					CH_2		
		1,1	5	9	13	OAc	b'	15a	15b
VI	90	1.01 (s)	1.70 (s)	2.02 (d,l)* ¹	1.95 (br.s)	2.18 (s)	—	4.46 (dd)	4.82 (dd)
VII	200	1.01 (s)	1.69 (s)	1.94 (d,1.3)	1.79 (br.s)	2.12 (s)	—	4.29 (dd)	4.63 (dd)
VIII	200	1.01 (s)	1.68 (d,0.4)	1.93 (d, 0.7)	1.76 (t,0.8)	2.06 (s)	1.28 (d,6.8)	4.05 (dd)	4.39 (dd)
Compound	MHz	CH				Olefinic H			
		11	14	a'	b'	7	8	10	12
VI	90	4.14 (m of d)	3.34* ² (m)	—	—	6.38 (d)	6.07 (d)	5.46 (d)	5.62 (br.s)
VII	200	3.55 (t?)	2.65* ² (br.s)	3.11 (dd)	3.31 (dd)	6.18 (d)	5.97 (d)	5.01* ² (d)	5.38 (br.s)
VIII	200	3.46* ² (t?)	≈ 2.5 (m)	2.55 (t?)	—	6.11 (d)	5.95 (d)	5.01* ² (d)	5.29 (br.s)
J (Hz)	7–8	10–11	11–12	14–15a 14–15b	15a–15b	11–a'	a'–b'	14–b'	
VI	16	10	—	4, 8	12	—	—	—	
VII	16.2	10.0	≈ 0	2.9, 4.6	12.4	9.7	11.7	5.4	
VIII	15.8	9.9	≈ 0	2.6, 4.6	12.0	≈ 11	≈ 11		

*¹ Splitting pattern and J value in Hz. s singlet, d doublet, dd doublet of doublets, t triplet, t? triplet-like, m multiplet, br broad.

*² Irradiated proton in decoupling experiments.

Table 2. ^1H -NMR chemical shifts in CDCl_3 (δ ppm from internal tetramethylsilane) for kitols

Compound	MHz	CH_3							CH_2	CH	
		1,1	1',1'	5,5'	9	9'	13	13'	15,15'	11	14
BURGER's kitol ⁽²⁾	60	1.01	1.01	1.66	1.78	1.87	1.78	1.13	≈ 3.72	n.d.* ¹	n.d.
Kitol-A	200	1.01	1.01	{ 1.66 1.70	1.80	1.89	1.82	1.25	$\approx 3.62^{*2}$ (m)* ³	3.03* ² (d)	2.52* ² (m)
Kitol-B	200	1.01	1.25	{ 1.67 1.70	1.70+1.79+1.85			1.25	$\approx 3.84^{*2}$ (m)	3.06* ² (d)	2.56* ² (br)

Compounds	MHz	Olefinic H						
		7,8	7',8'	10	10'	11'	12	12'
BURGER's kitol	60	5.94	5.98	5.42 (d)	n.d.	n.d.	5.14 (d)	n.d.
Kitol-A	200	{ 5.93 6.04	{ 6.03 6.12	5.15* ² (d)	5.97 (d)	6.32* ² (dd)	5.36* ² (br.s)* ⁴	5.75 (d)
		(ABq,16)* ³ (ABq 16)						
Kitol-B	200	5.98 (s?)	6.08 (s?)	5.09* ² (d)	5.98 (d)	6.29* ² (dd)	5.26* ² (br.s)	5.63 (d)

J (Hz)	10-11	11-12	11'-12'	10'-11'
BURGER's kitol	6	10.5		
Kitol-A	9	≈ 0	15	11
Kitol-B	10	≈ 0	15	11

*¹ n.d.=no datum.*² Irradiated proton in decoupling experiments.*³ Splitting pattern and J value in Hz. s singlet, s? singlet-like, d doublet, dd doublet of doublets, t triplet, q quartet, m multiplet, br broad.*⁴ Although a weak splitting (≈ 4 Hz) was observed near the top of the signal, the line-shape remained unchanged even if the neighbouring 11-H was irradiated.

^{13}C -NMR in CDCl_3 , δ 130.30 (8'-C), 137.86 (8-C), 21.96 (9'-Me) and 12.98 (9-Me) ppm.

Experimental

Spectra

Ultraviolet spectra (UV) were determined for solutions in ethanol and main λ_{max} is written in italics, if necessary. ^1H -NMR were run on a 200 MHz Varian XL-200 or on a 90 MHz NEVA-NV21 spectrometer for CDCl_3 solutions, and ^{13}C -NMR on a 22.6 MHz NEVA-NV21 spectrometer for CDCl_3 solutions. Mass spectra (MS) were obtained with a double focus high resolution spectrometer JEOL JMS-01SG equipped with a direct-inlet system using electron impact ionization (75 eV).

Isolation and Purification of Kitols

Isolation was conducted primarily according to the method of TAWARA *et al.*⁽¹⁷⁾ Whale *Physeter catodon* and *Balaenoptera edeni* liver was homogenized in a Waring blender for 1 h with methanol-chloroform (2: 1) (1.3 l/1 kg liver), the homogenate being filtered, concentrated and extracted eight times with chloroform. A concentrated extract

was then saponified under stirring with 20% KOH in ethanol for 3 h at room temperature and for 30 min at 45°C, followed by extraction with ether (eight times). To the non-saponifiable material from the ether extract, petroleum ether was added and extracted eighteen times with a half-volume of 90% methanol. The upper layer exhibited its λ_{max} at 290 nm, while the lower at 325 and 313 nm. Though further high-performance liquid chromatographic separation (μ -Porasil, 10% tetrahydrofuran in hexane) of components in the former fraction was unsuccessful, a pTLC on a silica-gel plate (0.25 mm-thick, developed with 20% acetone in benzene) gave well-defined two zones (Rf 0.51 and 0.45) containing kitol-A and -B, respectively. Purified kitols were obtained in poor yields (*ca.* 0.001–0.0001%).

Kitol-A (III)

UV 290 and 228 (infl.) nm; MS (sample temp. 200°C) M^+ ?, m/e 286 ($\text{M}^+/2$?, base), 271, 268, 255 and 199; ^1H -NMR see Table 2; ^{13}C -NMR δ ppm 12.77+12.83 (q; 9,9'-Me), 19.37 (t; 3,3'-C), 21.72 (q; 5,5'-Me), 22.01 (13-Me?), 29.00 (q; 1,1,1', 1'-Me), 29.30, 29.72 (13'-Me?), 33.05 (t; 4,4'-C), 34.28 (s; 1,1,1', 1'-C), 39.74 (t; 2,2'-C),

44.19 (13'-C?), 46.35, 47.64, 63.94, 65.47, 124.93 (10'-C+10-C?+12-C?), 126.20 (7,7'-C), 127.60 (5,5'-C), 130.19 (10'-C), 130.86 (12'-C?), 131.64 (13-C?), 137.71 (9,9'-C) and 137.85 (6,6', 8,8'-C).

Diacetate, MS (sample temp. 200°C) M^+ m/e 656.479 (calcd. for $C_{44}H_{84}O_4$, 656.481), m/e 596 (M-HOAc), 536 (M-2HOAc), 480, 422, 407, 404, 368, 344, 343, 328 ($M^+/2$, base), 268 (328-HOAc), 255 (328-CH₂OAc), 253 (328-HOAc-Me) and 199.

Kitol-B (9'-monocis-III)

UV 286 and 230 (infl.) nm; MS (sample temp. 200°C) M^+ ?, m/e 368, 286 ($M^+/2$?, base), 271, 268, 255, 223 and 199; ¹H-NMR see Table 2; ¹³C-NMR δ ppm 12.98 (9-Me), 21.96 (9'-Me), 130.30 (8'-C) and 137.86 (8-C). *Diacetate*, MS (sample temp. 200°C) M^+ m/e 656.484 (calcd. for $C_{44}H_{84}O_4$, 656.481).

Retinyl Acetate-Tetracyanoethylene Adduct (VI)

The adduct was prepared according to the previous report.¹⁰ Mp. 229°C, ¹H-NMR see Table 1.

Retinyl Acetate-Fumaronitrile Adduct (VII)

A mixture of fumaronitrile (*trans*-1,2-dicyanoethylene) (0.75 g) and retinyl acetate (2.52 g) in toluene (30 ml) was heated in a sealed tube at 150°C (bath temp.) for 4 h. After evaporated the solvent, the residue was purified through an alumina column (deactivated with 5% H₂O) employing 3% acetone in benzene and was recrystallized from benzene-ether. Yield 0.66 g. Rf (TLC, developed with ether-hexane 1:1) 0.38; mp. 154°C; UV 265 (ϵ 22,000) and 235 (ϵ 23,000) nm; IR (CHCl₃) 2250 (CN), 1740+1230+1050 (OAc) and 970 (*trans*-CH=CH-); MS (sample temp. 130°C) M^+ m/e 406.262 (65%) (calcd. for $C_{28}H_{34}N_2O_2$, 406.262), 391 (M-15, 13%), 347 (M-AcO), 346 (M-HOAc, 10%), 333 (M-CH₂OAc), 331 (M-15-HOAc), 328 (M-[NCCH=CHCN]), 189 (M-side chain $C_{14}H_{21}$, 23%) and 119 (100%); ¹H-NMR see Table 1.

Retinyl Acetate-Crotononitrile Adduct (VIII)

A mixture of crotononitrile (1.02 g) and retinyl acetate (0.5 g) in xylene (15 ml) was heated in a sealed tube at 170°C (bath temp.) for 7 h. After

evaporated the solvent, the residue was divided into two components on a pTLC plate. The upper band: UV 271 and 241 nm; ¹H-NMR see Table 1. The lower band: UV 271 and 238 (infl.).

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