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Quinones and Related Compounds in Higher Plants. XXI.$^{1)}$ New Findings on the Proton and Carbon-13 Nuclear Magnetic Resonance Spectra of Shikonin

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Inspection of the proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra of shikonin (**1**), cycloshikonin (**9**) and their derivatives led to the conclusion that, in solution, they exist neither as a semihydroquinone structure nor as tautomeric structures as reported recently, but as the commonly accepted 2-substituted 5,8-dihydroxy-1,4-naphthoquinone structure. Further, these compounds were shown to assume a comformation which allows the C-3 hydrogen and the *O*-functional group of the side chain to be in close proximity.

Keywords——shikonin; acetylshikonin; deoxyshikonin; cycloshikonin; tautomerism; ^{1}H-NMR; ^{13}C-NMR

Although the structures of shikonin and its antipode alkannin, occurring in various Bignoniaceous plants, are known to be **1a** and **2a**,$^{2-4)}$ respectively, two proposals were recently made regarding their structures in solution. The one presented by Papageorgiou$^{5)}$ was based on the proton nuclear magnetic resonance (^{1}H-NMR) spectral findings that the signal pattern (singlet at δ 7.17) of three aromatic protons of alkannin (**2**) differed from those observed in 2-substituted 1,4-naphthoquinones, *i.e.* acylalkannins including acetylalkannin (**3**) (a doublet ($J=1.1$ Hz) due to the C-3 proton at δ 6.96 and a singlet due to the C-6 and C-7 protons at δ 7.19) and deoxyalkannin (**4**) (a triplet ($J=1.1$ Hz) due to the C-3 proton at δ 6.83 and a singlet due to the C-6 and C-7 protons at δ 7.19). This difference was suggested to be attributable to the disturbance of the electronic equilibrium of the quinonoid ring of **2** through the hydrogen bond between the hydroxy group of the side chain and C-1 carbonyl group. Therefore, alkannin was presumed to exist predominantly in a semihydroquinone structure (**2b**). The other proposal, by Sankawa *et al.*,$^{6)}$ was based on the view of Moore and Scheuer$^{7)}$ that the appearance of the ^{1}H-NMR signals of all carbon-linked protons of naphthazarin (**5**) as a sole singlet at δ 7.13 can be explained in terms of rapid tautomerism of the naphthazarin system (**5a**, **5b**, **5c** and **5d**) in solution. In an extension of this view to alkannin (**2**) and cycloalkannin (**6**), the former being said to show two singlets due to tautomeric quinonoid protons at δ 7.04 and 7.12 and the latter at δ 7.12 and 7.14,$^{8)}$ it was assumed that both quinones **2** and **6** also exist as such tautomers.

By measurement of the 200 MHz ^{1}H-NMR spectrum (CDCl$_3$) of shikonin (**1**), however, we have ascertained that its C-3 proton appears as a sharp doublet ($J=1.1$ Hz) at δ 7.16, whereas the C-6 and C-7 protons appear as a singlet at δ 7.19. Based on decoupling experiments, the doublet nature of the former signal was further ascribed to an allylic coupling of the C-3 hydrogen with the proton on the hydroxy-bearing C-11.$^{9)}$ It was therefore assumed that, contrary to the aforementioned proposals, **1a** represents the preferred structure

TABLE I. ^{1}H-NMR Data for Shikonin Derivatives[a]

	1	1[b]	4	8	9	9[b]	10	11	12
H-3	7.16 d (1.1)	7.19 d (1.5)	6.84 t (1.1)	6.99 d (1.1)	7.21 d (1.8)	7.07 d (1.5)	7.23 br s	7.41 br s	7.06 br s
H-6	7.19 s	7.29 s	7.20 s	7.18 s	7.25 s	7.13 s	7.13 s	7.09 s	7.081 s
H-7	7.19 s	7.29 s	7.20 s	7.18 s	7.25 s	7.13 s	7.13 s	7.09 s	7.084 s
H-11	4.91 ddd (1.1, 4.4, 7.3)	5.00 dddd (1.5, 4.0, 4.8, 7.0)	2.64 br t (7.3)	6.02 ddd (1.1, 4.8, 7.0)	5.14 dd (1.8, 7.0)	5.07 dd (1.5, 6.6)	6.02 br t	5.14 br t	2.25—2.62 m
H-12	2.35 br td (7.3, 14.7)	2.31 br td (7.3, 14.7)	2.30 br q (7.3)	2.47 td (7.3, 14.7)	2.55—2.73 m	2.49—2.65 m	2.27—2.65 m	1.85 br s	
H-12	2.65 ddd (4.4, 7.3, 14.7)	2.55—2.67 m		2.63 ddd (4.8, 7.3, 14.7)	1.73—1.94 m	1.81—1.94 m			
H-13	5.20 qut (1.1, 7.5)	5.32 qut (1.5, 7.3)	5.18 qut (1.5, 7.0)	5.12 qut (1.5, 7.3)			5.02 qut (1.5, 7.3)		5.13 qut (1.5, 7.1)
H-13	—	—	—	—			—		—
H_3-15	1.65 br s	1.58 br s	1.60 br s	1.63 br s	1.36 s	1.33 s	1.53 br s	1.34 s	1.55 br s
H_3-16	1.76 br s	1.67 br s	1.70 br s	1.70 br s	1.38 s	1.37 s	1.66 br s	1.38 s	1.69 br s
OH-5	12.48 s	12.46 s	12.47 s	12.42 s	12.50 s	12.42 s	—	—	—
OH-8	12.58 s	12.55 s	12.62 s	12.58 s	12.51 s	12.42 s	—	—	—
OH-11	—	4.53 d (4.8)	—	—	—	—	—	—	—
Alc. OAc	—	—	—	2.14 s	—	—	2.04 s	—	—
Phen. OAc	—	—	—	—	—	—	2.375 s 2.384 s 2.40 s 2.42 s	2.36 s 2.37 s 2.38 s	2.37 s 2.40 s

a) The spectra were run at 200 MHz in $CDCl_3$ unless otherwise specified, with Me_4Si as an internal standard. Symbols are as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; qu, quintet; m, multiplet. Figures in parentheses are coupling constants in Hz. b) Measured in acetone-d_6.

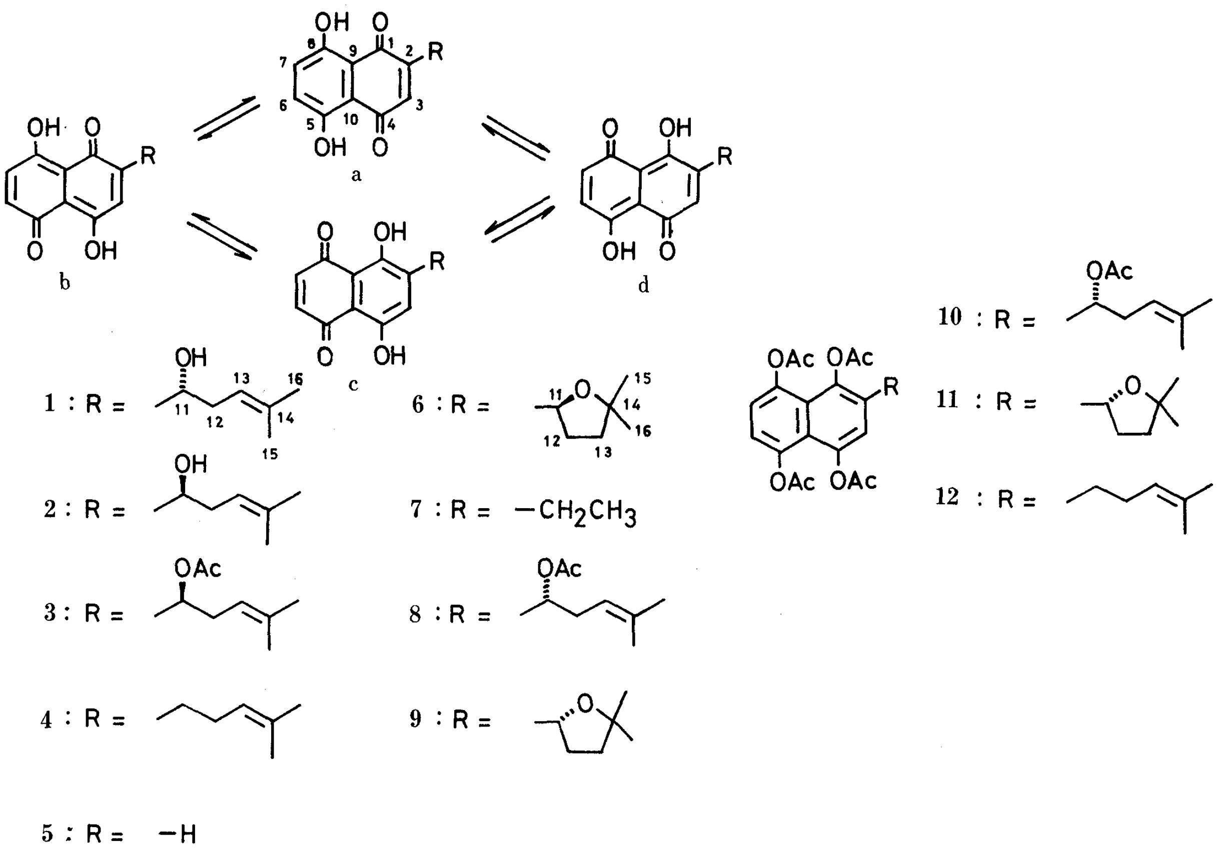

TABLE II. ^{13}C-NMR Data for Shikonin Derivatives

No. of carbon	Compound 1	 4	 8	 9
1	179.66 s	182.83 s	176.65 s	181.27 s
2	151.47 s	151.45 s	148.34 s	152.93 s
3	131.91 d	134.49 d	131.50 d	131.74 d
4	180.44 s	182.83 s	178.13 s	182.19 s
5	165.73 s	163.10 s	167.60 s	164.05 s
6	132.35 d	131.84[b] d	132.91[d] d	131.33[f] d
7	132.35 d	130.87[b] d	132.74[d] d	131.23[f] d
8	165.09 s	162.44 s	167.06 s	163.54 s
9	112.09[a] s	111.97[c] s	111.90[e] s	112.11[g] s
10	111.60[a] s	111.72[c] s	111.63[e] s	111.65[g] s
11	68.43 d	26.61 t	69.55 d	74.39 d
12	35.73 t	29.70 t	32.91 t	33.47 t
13	118.54 d	122.40 d	117.73 d	38.48 t
14	137.27 s	133.59 s	136.10 s	82.10 s
15	18.07 q	17.78 q	17.95 q	27.83 q
16	25.91 q	25.64 q	25.76 q	28.68 q
Acetyl CO			169.69 s	
Acetyl CH$_3$			20.94 q	

a—h) Values with the same superscript may be interchanged.

of shikonin in solution, which is in line with the original view of Moore and Scheuer[7] that the principal tautomer of 2-ethylnaphthazarin (**7**) in solution is **7a**.

Subsequently, in order to rationalize the unusual low field shift of the C-3 proton of **1**, the ^{1}H-NMR spectra of congeneric quinones, deoxyshikonin (**4**), acetylshikonin (**8**) and cycloshikonin (**9**), were also inspected. It was found that the C-3 protons of **1**, **8** and **9**, each having a C-11 *O*-functional group, resonate 0.32, 0.15 and 0.37 ppm downfield as compared with the signal (triplet ($J=1.1$ Hz) at δ 6.84) of **4**, whereas two aromatic hydrogens at C-6 and C-7 of the four quinones have essentially the same chemical shifts (δ 7.18—7.25) (Table I). Thus, the low field shift of the C-3 protons of the quinones **1**, **8** and **9** was interpreted in terms of the deshielding effect of the C-11 *O*-function. This explanation was further supported by the evidence that the C-3 protons of the leucoacetates **10** and **11** (δ 7.23 and 7.41) derived from the quinones **1** and **9** resonate 0.17 and 0.35 ppm downfield, respectively, relative to the corresponding proton (δ 7.06) of the leucoacetate **12** of deoxyshikonin (**4**). These results lead to an unequivocal conclusion that shikonin (**1**) exists in solution as the 2-substituted 5,8-dihydroxy-1,4-naphthoquinone structure **1a** rather than as the structure **1b** or the tautomeric structures **1a**, **1b**, **1c** and **1d**, and also that it assumes a conformation allowing the C-3 hydrogen to be in close proximity to the C-11 hydroxy group (within deshielding range). This is also the case for cycloshikonin (**9**). The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral data for **1**, **8** and **9** (Table II) are also in accord with this conclusion.[10] Naphthazarin (**5**), the basic skeleton of the above quinones, is reported to show only one signal at δ 173 due to carbonyl carbons and hydroxy bearing carbons. This finding indicates rapid equilibration between its tautomeric structures (**5a**, **5b**, **5c** and **5d**).[11,12] The spectra of shikonin (**1**), acetylshikonin (**8**) and cycloshikonin (**9**), however, show signals of carbonyl carbons between δ 176—182 and those of hydroxy-bearing aromatic carbons around δ 165, ruling out the tautomeric structures.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H-NMR spectra and ^{13}C-NMR spectra were measured on a JEOL-FX-200 spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard. The following materials were used for NMR measurements.

Shikonin (**1**), mp 143—144 °C, $[\theta]_{258}$—$[\theta]_{308}=7750$, containing 19% alkannin (**2**).

Deoxyshikonin (**4**), mp 90—91 °C, was isolated from cell cultures (M-231a strain) of *Lithospermum erythrorhizon* SIEB. *et* ZUCC. (Boraginaceae).[13]

Acetylshikonin (**8**), mp 108—109 °C, $[\theta]_{352}-[\theta]_{467}=8580$, was isolated from cell cultures of *Echium licopsis* L. (Boraginaceace) and was optically pure.[14]

Cycloshikonin (**9**), mp 87—88.5 °C, was derived from the above shikonin (**1**).[14]

Shikonin leucoacetate (**10**), mp 152 °C, deoxyshikonin leucoacetate (**12**), mp 146—148 °C, and cycloshikonin leucoacetate (**11**), mp 222—224 °C were prepared in the usual way from the above compounds **1**, **4** and **8**, respectively.

References and Notes

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8) They measured the spectrum of **2** in acetone-d_6 and that of **6** in $CDCl_3$.
9) The NMR spectra of **1** in $CDCl_3$ solution showed no significant shift of the signals of the C-3, C-6 and C-7 protons on varying the concentration or on the addition of a trace of HCl. On the other hand, in acetone-d_6 solution, a doublet ($J=1.5$ Hz) due to the C-3 proton at δ 7.19 and a singlet due to the C-6 and C-7 protons at δ 7.29 were observed.
10) Papageorgiou obtained essentially the same values for alkannin derivatives. *Cf.* V. P. Papageorgiou, *Planta Med.*, **40**, 305 (1980). We assigned the two quinone carbonyl signals of shikonin (**1**) based on the fact that the C-1 with a higher chemical shift shows long range coupling ($^3J_{C\text{-}H}=9.8$ Hz) with the C-3 hydrogen.
11) It was confirmed that the quinone **5** exists at low temperature (−160 °C) as a tautomeric structure **5a** (=**5c**) by means of ^{13}C-NMR measurement in the crystalline state. *Cf.* W.-I. Shian, E. N. Duesler, I. C. Paul, D. Y.

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