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Synthesis of 8-*O*-alkylshikonin(alkannin)s: new ketal formation, tautomerism, and nucleophilic aromatic substitution

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Abstract—The reaction of 5,8,1′-tri-*O*-acetylshikonin(alkannin) with KOH in an alcohol gives selectively 1′-*O*-acetyl-8-*O*-alkylshikonin(alkannin)s through methoxylation under tautomerism followed by quinone monoketalization.

As a part of our synthetic study to find new bioactive polyphenol–carbohydrate conjugates,[1] we have undertaken to prepare a variety of shikonin(alkannin) derivatives. Shikonin and alkannin are folk medicines of plant origin obtained from *Lithospermum erythrorhizon* and *Alkanna tinctoria*, respectively, for treatment of wounds.[2] Recently, biological activities such as anticancer,[3] antibacterial[4] (against MRSA), antiviral,[5] antifungal,[5] antiinflammatory,[6] inhibition of platelet activation,[7] inhibition of DNA-topoisomerase I,[8] induction of topoisomerase II-mediated DNA cleavage,[9] angiogenesis inhibition,[10] etc. have been reported (including simple derivatives).

During the course of our study, we have found that 5,8,1′-tri-*O*-acetylshikalkin[2,11] (**3**), on treatment with alkaline (NaOMe, NaOH, KOH, or K_2CO_3) methanol to remove selectively the phenolic acetyl groups, gave an unexpected product having a phenolic mono-*O*-methyl group (^{1}H NMR in $CDCl_3$: δ 3.98). As this product (later determined to be **5**) was produced almost instantaneously but was contaminated with several by-products, we chose, after many trials (to improve the yield and to purify the product), to carry out the reaction (15 min, rt) in milder toluene–MeOH (400:10 v/w for **3**)–KOH (4 equiv. for **3**; the mixture was sonicated for a while); if performed in a more concentrated solution (for example 40:1), the yield (and purity) of **5** fairly decreased, which suggests that the reaction intermediate is very reactive and reacts with **3** or mutually. Compound **5** could be purified by silica gel chromatography using a slightly acidic solvent (100:0.3 toluene–acetic acid), or better, by reversed-phase chromatography[12] (2:1 CH_3CN–H_2O) (yield, 40∼60%). Other separation procedures tried all failed.

When the above reaction was stopped before acidification [the solution was only washed with aq. 10%

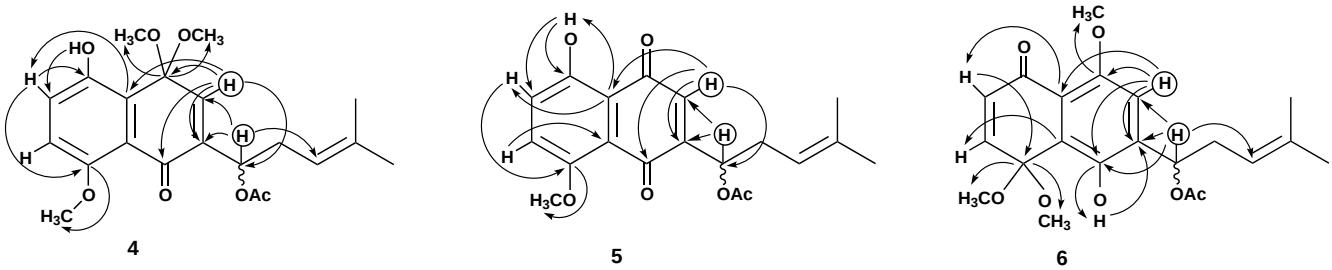

Figure 1. HMBC correlation for **4**, **5**, and **6** (circled H is a key hydrogen).

Keywords: shikonin; alkannin; quinone monoketal; tautomerism; nucleophilic aromatic substitution.

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$KHSO_4$[13] and water, and dried (Na_2SO_4)], a mixture of two new products (but not **5**) having ketal structures **4** and **6** were produced in high yield in a ratio of ~45:55 (~90%; however, these could not be purified due to their unstable character). However, on acidification [0.2 M HCl in THF–H_2O (2:1), 30 min], they degenerated to give a single product **5** (syrup, 78% based on **3**). This transformation of isonaphthazarin[14] to the naphthazarin structure (5,8-dihydroxy-1,4-naphthoquinone) (**6**→**5**) could be best explained by ready tautomerization[2,15,16] of the naphthazarin nucleus.

The structures of **5**, **4**, and **6** were determined to be 1′-*O*-acetyl-8-*O*-methylshikalkin[17] (**5**), 1′-*O*-acetyl-8-*O*-methyl-4-dimethylketal[18] (**4**), and 1′-*O*-acetyl-4-*O*-methyl-8-dimethylketal[19] (**6**) (having an isonaphthazarin structure), respectively, based on the NMR spectra (^1H,[15] ^1H–^1H COSY, ^{13}C, HMQC, and HMBC in $CDCl_3$) (see Fig. 1 and Table 1). The presence of MeO-8 in **5**, for example, was clearly shown by the HMBC spectrum ($CDCl_3$): the H-3 (δ 6.76) coupled to C-4a (δ 114.64) and the C-4a coupled to both phenolic *H*O (δ 12.43) and one of the protons (δ 7.29) in the benzene nucleus. This indicates, together with other data, that phenolic OH locates at C-5 and, therefore, the MeO group at C-8.

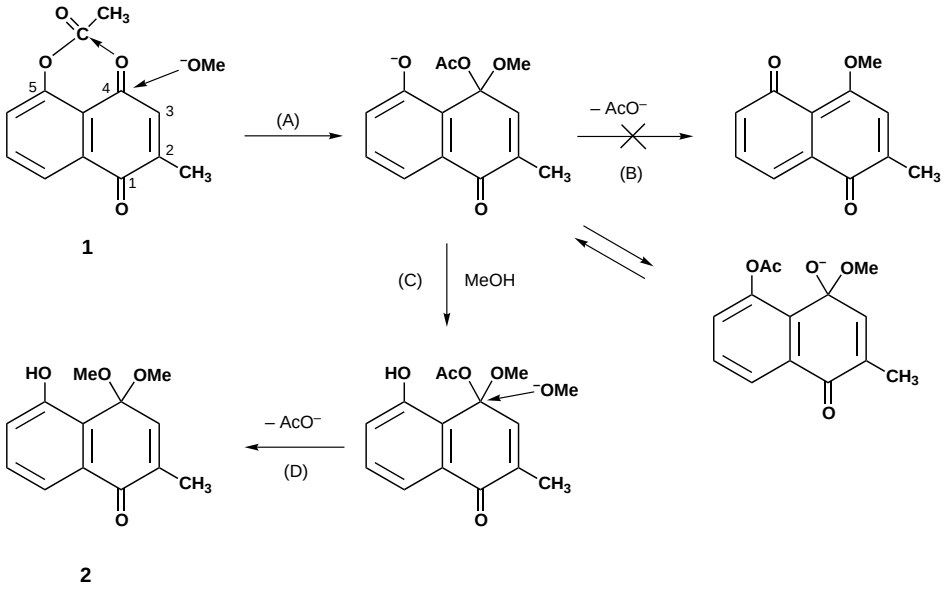
At this stage, 5-*O*-acetylplumbagin[20] (**1**) having a structure close to that of shikalkin but lacking the 8-hydroxy group was subjected to the same reaction, whereupon, however, no 5-methoxylation occurred but ketalization did occur at C-4 giving **2**[21] (an amorphous solid, 80%). As plumbagin itself gave no reaction under the same reaction conditions, presence of a phenolic acetoxy group at the *peri*-position of the quinone carbonyl will be necessary to give the ketal. A proposed mechanism of 4-ketalization of **1** by participation of the 5-*O*-acetyl group is shown in Scheme 1.

The mechanism of formation of **5** through **4** is described next. We propose (see Scheme 2) that this reaction occured through initial formal exchange of the oxo-1 and AcO-8 groups of **3** (to give I, although **3** does not contain I, NMR spectrometrically) followed by methoxylation at C-8 (to give II) and subsequent ketal formation at C-4 (to give **4**) according to the same mechanism, as shown in Scheme 1; finally, acidification of **4** gave the 8-methoxy derivative **5**. Direct attack of an MeO^- anion at C-1 of **3** to give III through a similar route will be hindered by the bulky 2-substituent. However, an alternative mechanism giving II (see Scheme 3) involving 8-methoxylation and subsequent deacetylation [that is, nucleophilic aromatic substitution[22,23] (S_NAr)] aided by participation of the AcO-1′ group will not be ruled out. Usually, S_NAr in a benzene ring occurs at the functional groups located at the *ortho* or *para* positions of strongly electron-withdrawing (EW) groups such as NO_2. In arene–metal complexes,[24] S_NAr also occurs due to the strongly EW property of metals. However, in our case of **3**, no such typical situation was envisaged at first glance. We consider, however, that the reaction could be accelerated by (1) the fusion of a strongly EW quinone ring; (2) presence of a slightly EW *para*-OAc group (compare the reaction for **1**) and (3) a conjugated system delocalizing the anion charge of the approaching MeO^- anion, as shown in IV. The real mechanism thus remains undetermined. Another reaction of **3** to give **5** through **6** is somewhat complicated

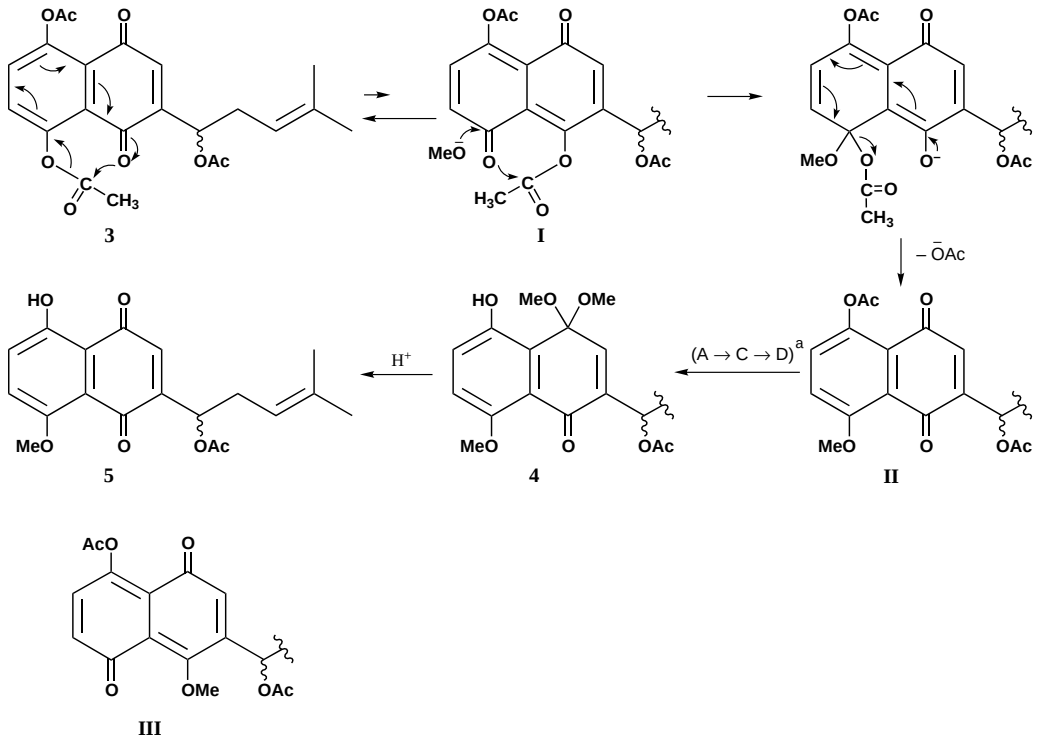
Table 1. ^{13}C NMR chemical shifts (ppm) of **2**, **4**, **5**, and **6** together with Shikalkin ($CDCl_3$, TMS, 125 MHz)

Carbon	**2**	**4**	**5**	**6**	Shikalkin[c]
1	184.11	181.78	181.91[b]	146.14	179.78
2	141.02	144.06	152.00	135.59	151.46
3	137.91	134.27	131.38	112.82	132.35[d]
4	97.99	98.45	190.16[b]	153.96	180.56
4a	122.32	122.53	114.64	119.75	111.61
5	155.47	149.21	156.42	183.01	165.04
6	121.55	123.38	126.87	135.38	132.43[d]
7	130.89	115.85	123.46	138.97	131.91
8	118.85	154.51	154.34	98.66	165.65
8a	132.78	120.49	117.45	122.61	112.09
1′	16.18	70.94	70.16	70.27	68.43
2′		32.42	32.85	33.27	35.73
3′		118.54	117.95	118.41	118.51
4′		~135.5[a]	135.92	~135.5[a]	137.37
5′		17.94	18.04	17.78	18.09
		25.77	25.78	25.69	25.94
OCH_3		56.60	56.87	56.74	
$(OCH_3)_2$	51.63	51.57		51.63	
	51.63	51.73		51.85	
CH_3CO		21.01	20.98	21.09	
CH_3CO		169.68	169.69	169.83	

[a] Difficult to specify by HMBC due to other close peaks.
[b] In HMBC, cross peaks between H-6 and C-4 ($^4J_{H\text{-}6,C\text{-}4}$) and between H-7 and C-1 ($^4J_{H\text{-}7,C\text{-}1}$) were also observed.
[c] The shift data are almost in accord with those of Ref. 16.
[d] Interchangeable.

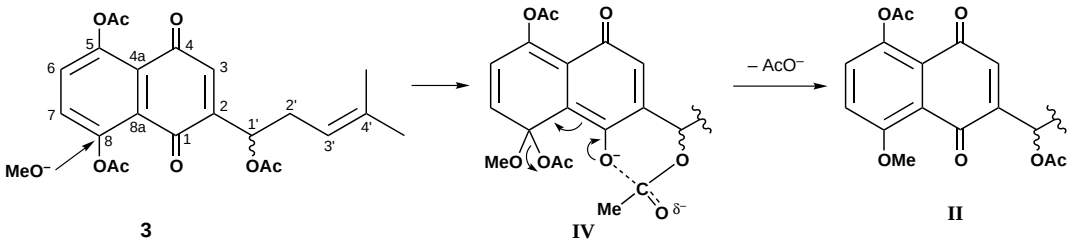


Scheme 1.

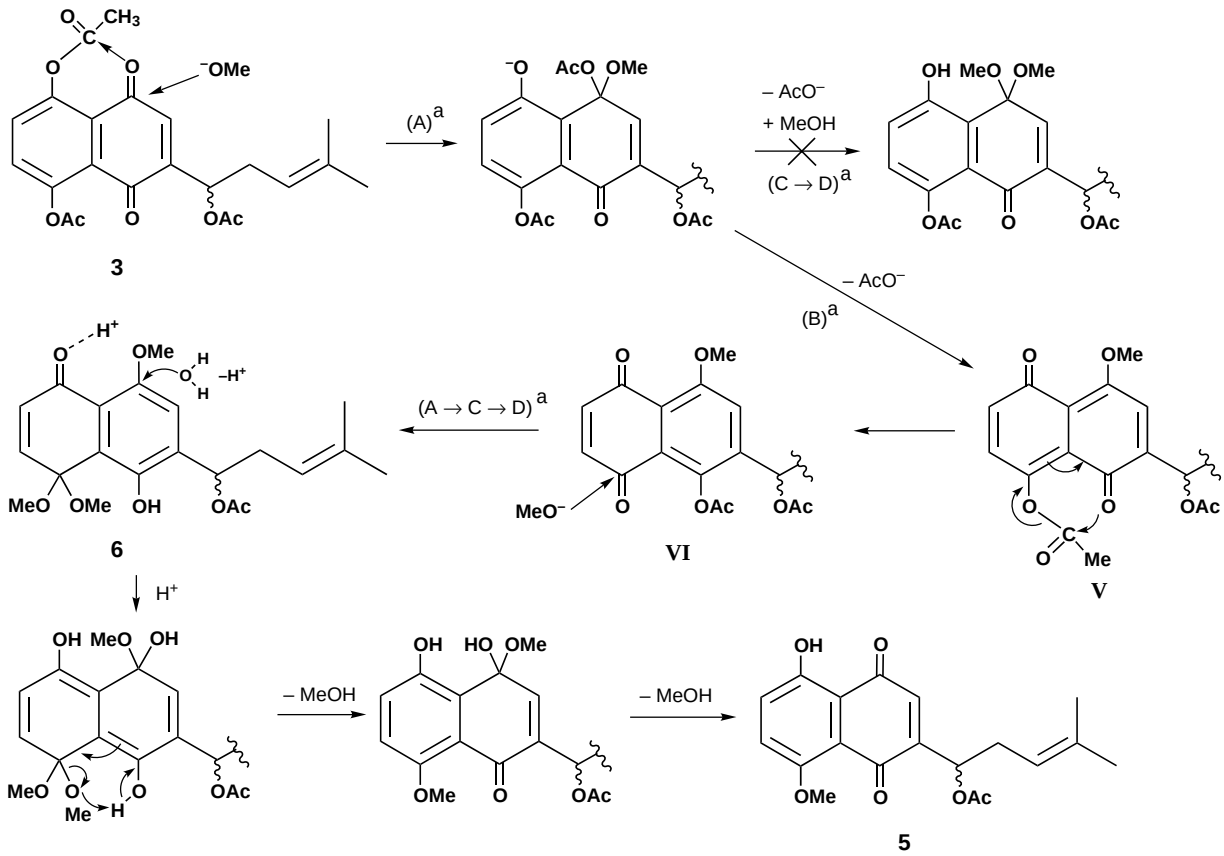


Scheme 2. [a]See Scheme 1.

(Scheme 4). It can be explained, however, that the methoxylation at C-4 of **3** first occurred (by the lack of a bulky substituent at C-3) and the 5-O^- anion formed degenerated, through V, to give VI via route B (not route C, possibly due to the EW AcO-8 group), and VI gave **6** by a similar sequence A→C→D, as shown in Scheme 1. Finally, the ketal **6** (with an isonaphthazarin structure) was hydrolyzed to give **5** through tautomeric rearrangement, in principle. The expected 1′-*O*-acetyl-4-*O*-methylisonaphthazarin was not observed. Other reaction mechanisms described here seem improbable, in our opinion. It should be stressed that, in all the reactions, methoxylation does not occur at C-3 of the quinone ring of **3**, being another nucleophile-accepting position.[25] Summarizing the above phenomena, it can be stated that the methoxylation first occurs at C-4 and C-8 of **3** for the C-4–C-4a–C-5 and C-8–C-8a–C-1 line-groups, respectively, but after alkaline ketalization, the ketals (**6** and **4**), on acidification, degenerate to a most stable 8-methoxy derivative (**5**).



Scheme 3.



Scheme 4. [a]See Scheme 1.

In other alcohols including secondary alcohols, **3** gave similar results fundamentally but not tertiary alcohols, which gave no reaction. 5,8-Di-*O*-acetylnaphthazarin and its analogs also gave the corresponding monoalkyl derivatives. All of these results will be reported elsewhere.

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13. If the solution was washed with aq. $NaHCO_3$ (instead of aq. $KHSO_4$) and dried (Na_2SO_4), a mixture of products (~78%) mainly composed of de-1′-*O*-Ac derivatives (A and B, respectively) of **4** and **6** were produced, which, in its ^1H NMR ($CDCl_3$) spectrum, showed a signal pattern similar to that of a mixture of **4** and **6** [H-3: δ 6.58 (d, A), 7.19 (br s, B), H-6 and −7: δ 7.08 (A), 6.54 (B) (each ABq), C(O*Me*)$_2$: δ 3.22–3.3, several peaks, ~6H in total, CO*Me*: δ 3.92, 3.93, ~3H in total].
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17. Compound **5**: ^1H NMR (500 MHz, $CDCl_3$): δ 1.58 and 1.69 [each 3H s, =C(CH_3)$_2$], 2.12 (3H, s, CH_3CO), 2.42 and 2.62 (each 1H ddd, H-2′a, 2′b), 3.98 (3H, s, CH_3O-8), 5.12 (1H, m, H-3′), 5.95 (1H, ddd, H-1′), 6.76 (1H, d, *J* 1.2 Hz, H-3); 7.29 (1H d, *J* 9.2 Hz, H-6) and 7.35 (1H d, *J* 9.2 Hz, H-7) forming ABq together, 12.43 (1H, s, HO-5); MS (FAB) *m*/*z* 345.11 $(M+1)^+$, 367.06 $(M+Na)^+$.
18. Compound **4**: ^1H NMR (500 MHz, $CDCl_3$): δ 1.57 and 1.66 [each 3H s, =C(CH_3)$_2$], 2.11 (3H, s, CH_3CO), 2.45–2.65 (2H, H-2′a, 2′b), 3.15 and 3.24 [each 3H s, (CH_3O)$_2$-4], 3.91 (3H, s, CH_3O-8), ~5.12 (1H, m, H-3′), 5.88 (1H, m, H-1′), 6.39 (1H, d, *J* 2 Hz, H-3); 7.03 (1H, d, *J* 9.2 Hz, H-7) and 7.12 (1H, d, *J* 9.2 Hz, H-6) forming ABq together, 7.54 (1H, s, HO-5); MS (FAB) *m*/*z* 391.15 $(M+1)^+$, 358.12 $(M-MeOH)^+$, 413.13 $(M+Na)^+$.
19. Compound **6**: ^1H NMR (500 MHz, $CDCl_3$): δ 1.51 and 1.66 [each 3H s, =C(CH_3)$_2$], 2.12 (3H, s, CH_3CO), 2.45–2.65 (2H, H-2′a, 2′b), 3.23 and 3.25 [each 3H s, (CH_3O)$_2$-8], 3.91 (3H, s, CH_3O-4), ~5.12 (1H, m, H-3′), 6.22 (1H, t, *J*~6 Hz, H-1′), 6.51 (1H, d, *J* 10 Hz, H-6) and 6.57 (1H, d, *J* 10 Hz, H-7) forming ABq together, 7.03 (1H, br s, H-3), 7.82 (1H, s, HO-1); MS: the same signals with those for **4**.
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