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Biosynthesis-Inspired Approach to Kujounin A_2 Using a Stereoselective Tsuji−Trost Alkylation

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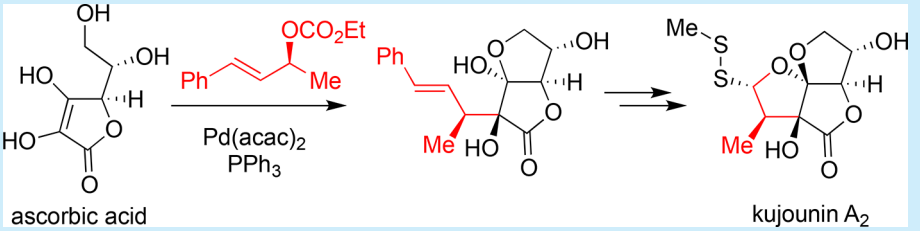
Supporting Information



ABSTRACT: A new biosynthesis was proposed for the kujounins A_1 and A_2 beginning from ascorbic acid, which in turn inspired a synthetic approach to kujounin A_2. The ring system was assembled in two steps using a stereoselective Tsuji−Trost reaction followed by ozonolysis. The chemically labile disulfide was introduced in several more steps. These results will make kujounin and its analogues available for further evaluation.

Allium species are commonly used as foods throughout the world and are reported to have beneficial health effects including anticancer activity and anti-inflammatory activity.[1] Sulfur compounds from allium have been found to show many pharmaceutical activities, including antibacterial activity,[2] inhibition of macrophage activation,[3] and inhibition of inflammation by blocking nuclear factor kappa B (NF-κB) activation.[4] Investigations of the chemistry of these plants have led to the discovery of a variety of small and reactive sulfur-containing natural products.[5] The Matsuda group from Kyoto recently reported the discovery of new natural products derived from *Allium fistulosum*, "Kujou", which is a cultivar of Welsh onion in Japan.[6] Kujounins A_1 (**1**) and A_2 (**2**) have an unusual tricyclic structure with a mixed disulfide as shown in Figure 1. Herein, we propose a new biosynthesis of these natural products and report the concise and stereoselective synthesis of kujounin A_2.

The tricyclic ring system of kujounin was proposed to originate with allicin via thioacrolein, which would combine with an unnamed monosaccharide and ultimately form the third ring by a possible radical process.[6] The proposal makes use of known allium organosulfur compounds but appears overly complicated. We were struck by the similarity between kujounins and the hongkonoids,[7] one of which is shown in Figure 1. The hongkonoids are described as ascorbylated terpenoids and were isolated from the plant *Dysoxylum hongkongense*, which has been used in Chinese herbal medicine.[7] The tricyclic ring in hongkonoid C (**4**) originates from ascorbic acid (**3**) and involves formation of a new carbon bond to C2 of the ascorbate structure. The presence of an almost identical tricyclic structure in kujounins suggests that they originate from ascorbic acid (**3**).

A new biosynthesis of kujounins is proposed in Figure 2. Six of the carbon atoms would arise with ascorbate (**11**) and three

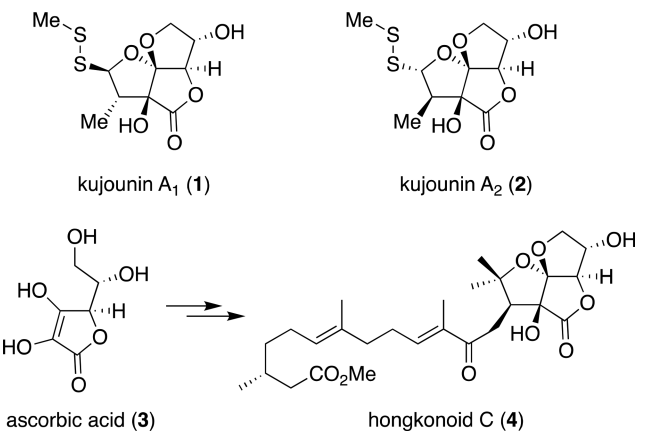


Figure 1. Structures of kujounin A_1 and A_2, hongkonoid C, and ascorbic acid (vitamin C).

from isoalliin, the stable precursor to many reactive sulfur compounds in allium species. Isoalliin is enzymatically activated to generate the unstable sulfenic acid **6**, which self-condenses with loss of water to form thiosulfinate **7**. Isoalliin is more common in onions than in garlic and gives rise to the lacrimatory factor, propanethial *S*-oxide, by the action of lacrimatory factor synthase on sulfenic acid **6**.[8] The methylthio group presumably arises from methiin (**8**) following a similar pathway, and exchange of the sulfinic acid fragments[9] would generate the mixed 1-propenyl methyl thiosulfinate **10**. Similar thiosulfinates have been directly detected in allium species.[10] Thiosulfinate **10** has all the component pieces, when combined with ascorbate **11**,

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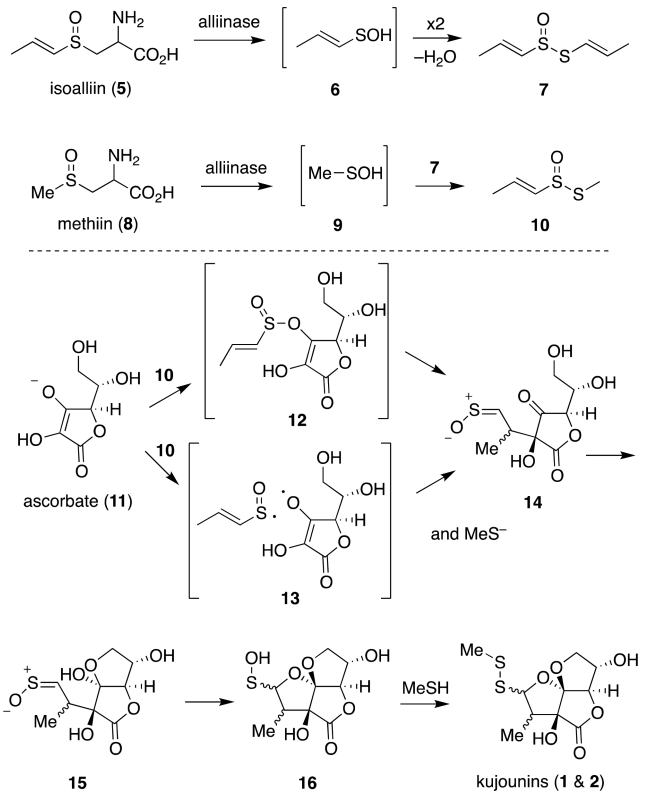
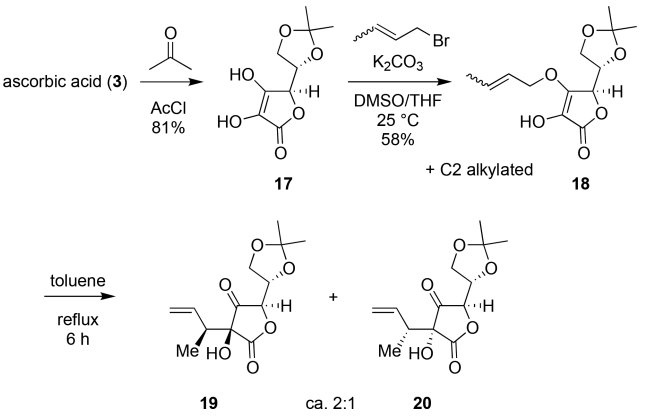


Figure 2. Proposed biosynthesis of kujounins from ascorbate (**11**) and isoalliin (**5**).

to make the kujounins. The key bond-forming step between C2 of the ascorbate and C2 of the 1-propene thiosulfinate **7** or **10** may proceed through one- or two-electron pathways. One speculative two-electron pathway would proceed via the sulfinic ester **12**, followed by a facile [3,3]-rearrangement[11] to forge the C−C bond. A possible one-electron pathway would proceed by electron transfer from ascorbate to one of the thiosulfinates, leading to the radical pair **13**, which would combine to form the C−C bond. Both routes would lead to the sulfine **14**, a reasonable intermediate for the kujounins. Stepwise cyclization of **14** to **16**, followed by coupling with the residual methanethiol, would lead to kujounins A_1 and A_2. The principle features of this proposed biosynthesis are the bond formation between ascorbate and 1-propene thiosulfinate at naturally reactive positions, the cascade cyclization to form the tricyclic structure, and the final incorporation of methanethiol in the disulfide.

The new biosynthesis suggests a retrosynthesis based on combining a three-carbon unit with ascorbic acid (**3**). The initial approach explored in our laboratory was to utilize a known Claisen rearrangement to introduce the C2 side chain stereoselectively.[12] The sequence is presented in Scheme 1. Ascorbic acid was protected as an acetonide. Alkylation with crotyl bromide (technical grade) led to the expected ether **18** along with some of the Z-ether and direct C-alkylation products.[13] The thermal rearrangement of **18** produced the C2-substituted products as a mixture of stereoisomers. As previously reported, the bond formation occurs at both the top and bottom faces of the enol ether in **18**. In addition, stereochemical mixtures at the methyl-bearing center were produced, arising from unselective rearrangement and the presence of the minor Z-isomer in precursor **18**. Compounds **19** and **20**, which were major components in the complex product

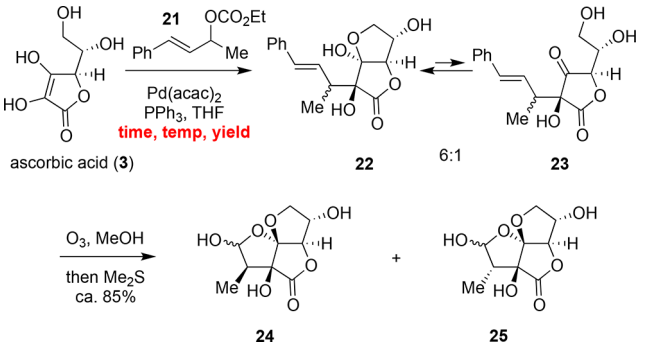
Scheme 1. Claisen Rearrangement To Introduce the C2 Carbon Chain for Kujounins



mixture, contain all of the atoms necessary to form kujounin A_2. However, the complex mixture of stereoisomers arising from this sequence and the difficulties encountered in attempting to separate them reduced our enthusiasm for this route. It is worth mentioning that the hongkonoid natural products were prepared using a similar Claisen rearrangement step. It also produced a stereochemical mixture, but the researchers were able to separate the isomers and complete the synthesis.[7]

A more satisfying route was based on diastereoselective Tsuji−Trost couplings, as shown in Scheme 2 and Table 1.[14,15]

Scheme 2. Tsuji−Trost Alkylation To Introduce the C2 Side Chains for Kujounin



Ascorbic acid is known to participate in Tsuji−Trost allylations efficiently.[16] The enantiopure allylic carbonates **21-R** and **21-S** were prepared by enzymatic resolution of the racemic alcohol

Table 1. Tsuji−Trost Conditions for the Synthesis of Styrene 22 (and Lactols 24 and 25) from 2 equiv of Ascorbic Acid[a]

entry	21	PPh_3 (mol %)	time (h)	T (°C)	yield[b] (%)	dr 24/25
1	*RS*	12	24	23	86	63:37
2[a]	*S*	20	5	70	85	77:23
3	*S*	25	24	23	86	82:18
4	*R*	20	70	23	91	54:46
5	*S*	12	24	0	40	99:01
6	*S*	12	24	0−23	91	90:10
7[c]	*S*	12	14	23	86	90:10

[a]All reactions used 3 mol % of $Pd(acac)_2$, except entry 2, which used 5 mol % of $Pd(acac)_2$. [b]The yield is based on carbonate **21**. [c]DMSO was used as the solvent in this entry.

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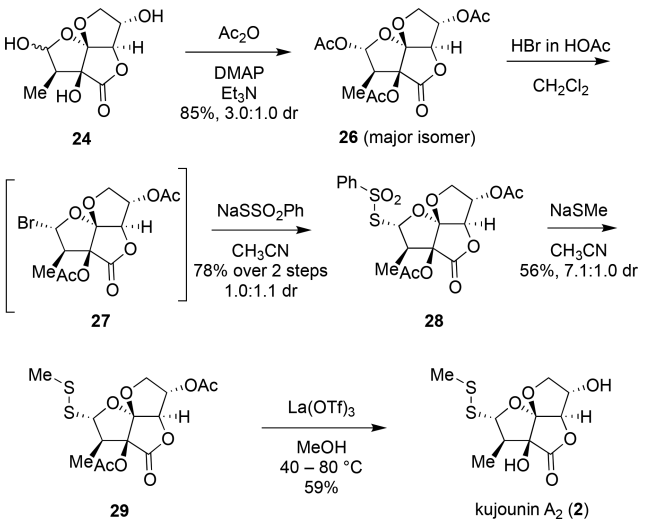
and derivatization.[17] These enantiopure substrates form Pd(II) intermediates with inversion and should subsequently react with the ascorbate nucleophile with inversion, leading to overall retention in the process.[17,18] The phenyl substituent was expected to control the regiochemistry of the substitution. The alkylation reactions worked well. Two equivalents of ascorbic acid was used to increase conversion. Compound **22** was formed from carbonate **21-S** with the methyl group forward as the major diastereomer. Keto-lactol isomers were observed: **22** and **23** were present in a 6:1 equilibrium mixture in the NMR samples, which complicated the analysis. The diastereomeric ratios were analyzed most easily after ozonolysis to the lactols **24** and **25**, and the results are presented in Table 1. Racemic carbonate **21** favored the β-methyl epimer **24** in a 63:37 ratio (entry 1). Using the enantiopure carbonate **21-S** favored formation of isomer **24**, as would be expected with a double-inversion mechanism for substitution. The substitution reaction was most selective at 0 °C (entry 5), but the yield was reduced. The reaction was less selective at higher temperatures (entries 2 and 3). Switching to enantiopure **21-R** under the standard conditions led to an approximately equal mixture of **24** and **25** (ratio 54:46, entry 4). The absolute configuration of the unsaturated carbonate **21** influences the stereoselectivity, but it does not dominate the outcome. Entries 6 and 7, with the latter using DMSO as the solvent, represent good compromises between selectivity and yield for the Tsuji–Trost coupling, and both conditions produce 90:10 mixtures and ca. 90% yields. Alkene **22** is crystalline, and diastereomerically clean samples are available by recrystallization. The Tsuji–Trost coupling was much more stereoselective than the Claisen method (Scheme 1) and allowed lactol **24** to be prepared with high diastereoselectivity and good yield.

The synthesis of kujounin A_2 was completed as outlined in Scheme 3. Lactol **24** contains all of the carbon atoms necessary

Scheme 3. Synthesis of Kujounin A_2



to complete the skeleton of kujounin. It was present as a 3:1 mixture of lactol isomers and was acylated to give triacetate **26**, which was separated from the anomeric isomer by silica gel chromatography. NOE studies confirmed the relative configuration of the methyl-bearing center. The major isomer of **26** was assigned as the α-acetate.[19] The mixture of anomeric isomers of **26** was treated with HBr in acetic acid/DCM to produce the reactive anomeric bromide **27**, which was isolated by extraction but could not be further purified. One major diastereomer of the bromide **27** was observed, and NOE studies were consistent with the α-bromide drawn. The sulfur was introduced by displacement with sodium phenylthiosulfonate to produce an anomeric mixture in which the desired α-thiosulfonate **28** was formed with a dr of 1.0:1.1 in 78% over two steps.[20,21] The sequence was also successful with the methylthiosulfonate, but the phenylthiosulfonate gave slightly higher yields and a better diastereomeric ratio.[21] The β to α selectivity varied from run to run but generally favored the β-isomer as one would expect from an S_N2 mechanism for the substitution of **27**. The desired α-isomer presumably arises from a more complex solvolysis and recombination sequence. The desired α-anomers of the disulfide structures **28**, **29**, and **2** all showed distinctive 10 Hz coupling constants at the anomeric proton, and this unique feature was used to assign the anomeric configuration in this series. Treatment of **28** with NaSMe produced the disulfide **29** in 56% yield but with surprisingly good 7.1:1.0 diastereoselectivity. Apparently the α-thiosulfonate **28** reacted selectively. Hydrolysis of the acetate esters with $La(OTf)_3$ in methanol gave synthetic kujounin A_2 as a single diastereomer in 59% yield.[22] Hydrolysis of the secondary acetate was fast, but the tertiary acetate hydrolyzed slowly. Unreacted tertiary acetate was recovered in 30% yield as a 2.5:1.0 dr favoring the α-disulfide. Hydrolysis of the tertiary acetate is diastereoselective, with the more accessible α-disulfide hydrolyzing more rapidly. The desired α-isomer of **28** proceeded to product through these last two diastereoselective steps, but the undesired β-isomer did not.

Synthetic kujounin A_2 showed spectroscopic properties consistent with those reported for the natural product. The measured optical rotation, $[\alpha]^{23}_D = -86.5$ (c = 0.40, MeOH), was consistent with that measured for the natural product: $[\alpha]^{25}_D = -74.1$ in MeOH. The overall synthetic sequence was short and effective.

We have completed a synthesis of kujounin A_2. The strategy was inspired by consideration of a new biosynthesis for the kujounin natural products. The ring system was constructed in two steps from ascorbic acid using a stereoselective Tsuji–Trost reaction and ozonolysis. The remaining five steps were used to introduce the sensitive disulfide functionality of the natural product. The biological activity of synthetic kujounin A_2 is being evaluated. This rapid synthesis of the kujounin ring system is suitable for the preparation of analogues.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b02530.

Experimental procedures, characterization, and ^{1}H and ^{13}C NMR spectra for all new compounds. DP4+ analysis for triacetates **26** major and **26** minor (PDF)

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Notes

The authors declare no competing financial interest.

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