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Introduction of the (−)-Berkelic Acid Side Chain and Assignment of the C-22 Stereochemistry

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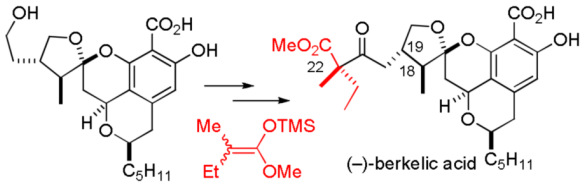
Abstract



A Kiyooka aldol condensation of an aldehyde with a trimethylsilyl ketene acetal and the oxazaborolidinone prepared from *N*-Ts-(*S*)-valine gives two of the four possible aldol adducts which were oxidized and deprotected to complete the synthesis of (−)-berkelic acid and (−)-22-*epi*-berkelic acid. This synthesis establishes the absolute stereochemistry and assigns the stereochemistry at C-22. A biosynthetic pathway is proposed that is consistent with the known absolute stereochemistry at the quaternary carbon of spiciferone A, spicifernin, and berkelic acid and provides a simple explanation for the differing stereochemistry at C-18 and C-19 of spicifernin and berkelic acid.

Introduction

Berkelic acid (**1**) and its congener, spiciferone A (**2**), were recently isolated by Stierle and coworkers from a *Penicillium* species obtained from the surface water of Berkeley Pit Lake, an abandoned open-pit copper mine (see Chart 1).[1] The acidic water (pH 2.5) is contaminated with metal sulfates at high concentrations so that only extremophiles, which may produce novel natural products, can survive. The structure of berkelic acid (**1**) was determined to be a novel spiroketal from the analysis of the NMR and mass spectral data of **1** and the analogous methyl ester. The relative stereochemistry of the side chain stereocenter (C-22) and the absolute stereochemistry were not assigned.

Berkelic acid (**1**) inhibits MMP-3 in the micromolar range (GI_{50} = 1.87 μM) and caspase-1 in the millimolar range (GI_{50} = 0.098 mM). Inhibitors that act by blocking the activity of proteolytic enzymes (MMPs) used by tumor cells to promote metastatic spread provide a new approach for cancer treatment. Berkelic acid was tested in the National Cancer Institute antitumor screen against 60 human cell lines and showed selective activity toward ovarian cancer OVCAR-3 with a GI_{50} of 91 nM.[1]

We hypothesized that **1** could be prepared by a short sequence starting from ketal aldehyde **4** and 2,6-dihydroxybenzoic acid **5** (see Scheme 1). Benzoic acid **5**, a synthetic, and possibly

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Supporting Information Available. Experimental procedures for reactions not in the experimental section, comparison tables S1–S2 of NMR data, and ^{1}H and ^{13}C NMR spectral data. Data for the acid prepared by hydrolysis of **8a**, **35**, and **36** in the NCI 60 cell screen. This material is available free of charge via the Internet at http://pubs.acs.org.

biosynthetic, precursor to pulvilloric acid (**6**), has been synthesized in both racemic[2] and optically pure form.[3] Berkelic acid (**1**) might be biosynthesized by an analogous coupling of **5** and a ketal aldehyde related to **4**. Alternatively, it is possible that berkelic acid (**1**) is biosynthesized by the nucleophilic addition of ketone **7** to pulvilloric acid (**6**). Ketal aldehyde **4** and ketone **7** are related to spicifernin (**3**) which occurs with spiciferone A (**2**) in *Cochliobolus spicifier*.[4]

In a model study,[5,6] we condensed **4a** with (±)-**5** in MeOH containing Dowex 50WX8-400-H^+ for 12 h at 25 °C to provide a mixture of the tetracyclic acids that was treated with diazomethane to give **8a** (24%) and **9a** (24%) and minor amounts of the other two diastereomers (see Scheme 2). The desired isomer **8a** was calculated to be 2.4 kcal/mol more stable than **9a** using MMX with conformational searching.[7] As expected, treatment of the mixture of four methyl esters with TFA in $CDCl_3$ afforded a mixture containing mainly **8a**, which was isolated in 50% yield from **5**.

For the synthesis of (−)-berkelic acid,[8] we prepared (*R*)-(−)-**5** and **4b** and condensed them in MeOH containing Dowex 50WX8-400-H^+ for 12 h at 25 °C to provide a mixture of the tetracyclic acids that was treated with diazomethane to give **8b** (29%) and **9b** (22%) and minor amounts of a third diastereomer. Equilibration with TFA in $CDCl_3$ had little effect, giving **8b** (39%) and **9b** (19%). As a result of the substituents on the tetrahydrofuran, the desired isomer **8b** was calculated[7,9] to be only 0.8 kcal/mol more stable than **9b**, which is consistent with the formation of a 2:1 mixture of **8b** and **9b** after equilibration.

At first this was of great concern, but the ^{1}H NMR spectral data of the desired product **8b** did not fit well with the data for berkelic acid methyl ester suggesting that the stereochemistry of berkelic acid had been assigned incorrectly. The trans stereochemistry at C-18 and C-19 appeared to be correctly assigned. The relative stereochemistry of these two centers relative to the anomeric center C-17 was assigned solely on the basis of an NOE from the methyl group (C-25) to H-16β and an H-20 (see Figure 1).[1] However, our MMX calculations indicated that this NOE is more consistent with structure **10**, which has the opposite stereochemistry to **1** at both C-18 and C-19, because structure **1** should have NOEs to both H-16β and H-16α. As this work was being completed, Fürstner reported a synthesis of the enantiomer of berkelic acid methyl ester and reassigned the stereochemistry at C-18 and C-19 as shown in **10**.[10]

We therefore prepared *ent*-**4b** and condensed it with (*R*)-(−)-**5** in MeOH containing Dowex 50WX8-400-H^+ for 60 h at 25 °C to provide a mixture of the tetracyclic acids corresponding to esters **11** and **12** (see Scheme 3).[8] Partial cleavage of the TBDPS group occurred because this reaction was slower than that with **4**, requiring 60 h for complete reaction. Esterification with diazomethane provided silyl ether **11** (30%) and alcohol **12** (22%) with no diastereomers formed at C-15 and C-17 before or after TFA equilibration. The selectivity is consistent with the calculated strain energies, which indicated that **11** is 3.9 kcal/mol more stable than the isomer analogous to **9b**. As we expected, the spectral data of **11** fit well with those of berkelic acid methyl ester suggesting that **10** is the structure of berkelic acid with the opposite stereochemistry at C-18 and C-19 to that originally proposed in **1** and that the stereochemistry at both C-15 and C-17 in the natural product is thermodynamically controlled.

Although the structure of **12** is very close to the revised structure of berkelic acid (**10**), the introduction of the five-carbon side chain, the assignment of the stereochemistry at C-22 and the choice of protecting group for the aromatic acid and phenol proved to be very challenging. We describe here the full details of the completion of the synthesis,[8] the assignment of stereochemistry at C-22, and an analysis of the biosynthesis of berkelic acid.

Results and Discussion

Protection of **11** with Ac_2O and pyridine afforded **13**, which was desilylated with TBAF/HOAc to give alcohol **14** in 74% overall yield from **11**. Dess-Martin oxidation of **14** gave aldehyde **15** in 87% yield. Attempted oxidation of phenol alcohol **12** with Dess-Martin periodinane resulted in decomposition, establishing that protection of the phenol is necessary.[11]

Unfortunately, addition of **15** to the enolate prepared by treatment of methyl 2-methylbutanoate with LDA at −78 °C destroyed the aldehyde and cleaved the acetate group, rather than forming the desired aldol adducts. These aldol conditions are successful with 3-phenylbutanal (83%) and citronellal (70%). Acidic Mukaiyama aldol conditions were equally unsuccessful: no aldol products were obtained from the $TiCl_4$-catalyzed reaction of **15** and trimethylsilyl ketene acetal **19**[12] (see Scheme 4) prepared from methyl 2-methylbutanoate, although aldol adducts (74%) were obtained from 3-phenylbutanal under these conditions. We needed to find a protecting group for the phenol that was compatible with the aldol reaction and to develop a procedure that utilized both TBDPS ether phenol **11** and hydroxy phenol **12**.

Desilylation of **11** with TBAF/AcOH in THF afforded alcohol **12** in 91% yield. Protection of both hydroxy groups in **12** with TBSOTf and 2,6-lutidine gave bis TBS ether **16**, whereas protection of **12** with TBSCl and imidazole in DMF selectively protected the primary alcohol. Treatment of bis TBS ether **16** with $Ce(OTf)_n \bullet xH_2O$ (19–23% Ce) selectively cleaved the alkyl TBS ether rather than the phenol TBS group to give alcohol **17** in 81% yield.[13] Oxidation of **17** with Dess-Martin periodinane afforded aldehyde **18**.

Unfortunately, addition of **18** to the enolate prepared by treatment of methyl 2-methylbutanoate with LDA at −78 °C gave no aldol products. The *N*-methylimidazole and LiCl-catalyzed Mukaiyama aldol reaction[14] proceeds under very mild conditions and appeared to be compatible with the functionality in **18**. We were pleased to find that reaction of **18** with trimethylsilyl ketene acetal **19** (10 equiv), LiCl (0.5 equiv), *N*-methylimidazole (0.25 equiv), and 4 Å molecular sieves in DMF afforded an inseparable mixture of all four aldol products, which was subjected to Dess-Martin oxidation to give an inseparable 1:1 mixture of keto esters **20** in 44% overall yield. Removal of the TBS group using CsF in MeOH afforded a 1:1 mixture of the methyl esters of berkelic acid and 22-*epi*-berkelic acid (**21**) in 88% yield. The ^{1}H NMR spectral data of **21** match those of berkelic acid methyl ester in $CDCl_3$ but there are doubled peaks for the C-20 methylene group and the methyl and ethyl groups attached to C-22 as expected, since **21** is a mixture of isomers at C-22. The correspondence of the ^{1}H NMR spectral data of **21** and natural berkelic acid methyl ester confirmed the reassignment of the stereochemistry at C-18 and C-19.

Selective cleavage of the methyl benzoate of **21** in the presence of the β-keto ester group is not possible, as was also observed by Fürstner,[10] so we needed to use a different protecting group for the carboxylic acid. The protection of the phenol by silylating both the alcohol and phenol and selective liberation of the alcohol is also cumbersome. We therefore decided to protect both the phenol and carboxylic acid with allyl groups as reported by Oikawa in his synthesis of prelasalocid.[15] Condensation of *ent*-**4b** and (*R*)-(−)-**5** with Dowex 50WX8-400-H^+ in MeOH for 60 h gave crude tetracyclic salicylic acids with a partially deprotected side chain. Protection of this mixture with allyl bromide and K_2CO_3 in DMF gave silyl ether **22** (32%) and alcohol **23** (20%) without allylation of the primary alcohol of **23** (see Scheme 5). Additional alcohol **23** was prepared by desilylation of **22** with TBAF/HOAc (86%) so that the overall yield of **23** from *ent*-**4b** is 48%. Aldehyde **24** was prepared in 88% yield by Dess-Martin oxidation of **23**.

Treatment of aldehyde **24** with trimethylsilyl ketene acetal **19**, LiCl, and *N*-methylimidazole afforded an inseparable mixture of all four possible aldol products, which was subjected to

Dess-Martin oxidation to give a 1:1 mixture of keto esters in 60% yield from **24**. Removal of both allyl groups[15] with $Pd(Ph_3P)_4$, Et_3N, and HCO_2H afforded **25** (1:1 mixture of berkelic acid and 22-*epi*-berkelic acid) in 81% yield. The ^1H and ^{13}C NMR spectral data of **25** match those of natural berkelic acid both in $CDCl_3$ and CD_3OD, but there are doubled peaks for the C-20 methylene group and the methyl and ethyl groups attached to C-22 in the ^1H NMR spectrum. The optical rotation of synthetic **25**, $[\alpha]\ D^{22} - 104.6$ ($c = 0.07$, MeOH), has the same sign as that of the natural berkelic acid, $[\alpha]_D^{22} - 83.5$ ($c = 0.0113$, MeOH),[1] suggesting that the absolute stereochemistry of natural berkelic acid is most likely as shown in **25**. The use of allyl protecting groups for both the phenol and carboxylic acid provides a very efficient solution of selectively protecting an acid and phenol without protecting a primary alcohol. The allyl groups also protect the phenol during the Dess-Martin oxidation of the C-21 alcohol to a ketone. In his synthesis of the methyl ester of berkelic acid, Fürstner reported that only the Swern oxidation (35%) could be used to oxidize the C-21 alcohol with a free phenol because other oxidants destroy the electron rich aromatic ring.[10]

We still needed to develop a procedure to either control the stereochemistry at the quaternary center[16] C-22, or at least allow us to separate the aldol products. Kobayashi reported the diastereoselective alkylation of the enolate of **26** (see Scheme 6).[17] Unfortunately, no aldol products were obtained from addition of the sodium enolate of **26** to the model aldehyde heptanal. TBS ketene aminal **27** undergoes a vinylogous Mukaiyama aldol reaction with hexanal at the γ-, not α-position so this reagent cannot be used.[18] We prepared **27** and hydrogenated it with 5% Pd/C under 1 atm of H_2 to give **28** stereospecifically as determined by an NOE between the methyl and TBS groups. We hoped that **28** would undergo a Mukaiyama aldol reaction now that a vinylogous Mukaiyama reaction was no longer possible. To our disappointment, attempted Mukaiyama aldol reactions of **28** with heptanal using either $TiCl_4$ at various temperatures, LiCl and *N*-methylimidazole, or TMSOTf and *i*-Pr_2EtN were unsuccessful.

Koga reported the highly enantioselective alkylation of α-alkyl β-keto esters to give α,α-dialkyl β- keto esters.[19,20] Chiral enamines were prepared from the α-alkyl β-keto esters and (*S*)-valine *tert*-butyl ester (**30**)[21] in the presence of a catalytic amount of BF_3•OEt_2. Metalation of the enamines with LDA, coordination of the lithium cations with an additive (HMPA or THF), alkylation of the enamides and hydrolysis of the resulting imines affords the α,α-dialkyl β-keto esters with >90% ee. β-Keto ester **29** was synthesized by Dess-Martin oxidation of **39** (see below) to test this approach. To our surprise, **29** didn't react with (*S*)-valine *tert*-butyl ester (**30**) to give enamine **31** under a variety of conditions that were successful in our hands with ethyl 2-methylacetoacetate and ethyl 2-oxocyclohexanecarboxylate as reported.[19,20] All of the reported examples of enamine formation from **30** use either cyclic β-keto esters or ethyl 2-methylacetoacetate, suggesting that our failure with **29** results from steric interactions between the bulky substituents of **29** and the valine moiety. This approach was therefore not pursued.

Kiyooka reported that stoichiometric oxazaborolidinone (*S*)-**32**, which is prepared *in situ* from *N*-Ts-(*S*)-valine[22] and BH_3•THF, induces aldol reactions between aldehydes and trimethylsilyl ketene acetals. Reaction occurs selectively at the *Si*-face of the aldehyde with excellent control of the stereochemistry at the β-carbon with the hydroxy group. Mixtures of isomers are formed at the α-carbon center.[23] We needed to control the stereochemistry at the α-carbon; the stereochemistry of the β-carbon with the hydroxy group is irrelevant since it is oxidized to a ketone. Although Kiyooka's protocol won't solve our stereoselectivity problem, the mildly acidic conditions should be compatible with our substrate and we may be able to separate the aldol products if only two, rather than four, isomers are formed.

As expected, reaction of **24** and **19** as described by Kiyooka using (*S*)-**32** afforded only **33** (40%) and **34** (40%) which were fortunately separable by preparative TLC, although at this

time we didn't know which isomer was which (see Scheme 7). To our surprise, the analogous reaction using (*R*)-**32** resulted in reduction of **24** to give primary alcohol **23**. This suggests that the stereochemical preferences of **24** and (*S*)-**32** are matched, while those of **24** and (*R*)-**32** are mismatched leading to reduction of the aldehyde instead of the desired aldol reaction. Very different yields have been previously observed with matched and mismatched aldehydes.[24]

Dess–Martin oxidation of the less polar isomer **33** afforded the keto ester in 85% yield which was deprotected with $Pd(Ph_3P)_4$, Et_3N, and HCO_2H to afford berkelic acid (**35**), although the stereochemistry at C-22 was still unassigned. The ^1H and ^{13}C NMR spectral data of **35** in both $CDCl_3$ and CD_3OD are identical to those of natural berkelic acid.[8] The optical rotation of synthetic berkelic acid (**35**), $[\alpha]_D^{22}$−115.5 (*c* = 0.55, MeOH), has the same sign as that of the natural product, $[\alpha]_D^{22}$ − 83.5 (*c* = 0.0113, MeOH), indicating that the absolute stereochemistry is as shown. The differing numerical values may result from the low sample concentration used for the natural product. A similar sequence from the more polar isomer **34** afforded 22-*epi*-berkelic acid (**36**). The spectral data of **36** are similar to those of berkelic acid, but there are significant differences, most notably in the ^1H NMR spectra for the C-20 methylene group and the methyl and ethyl groups attached to C-22. In $CDCl_3$, the chemical shifts of the two protons on C-20 of **36** (δ 2.90, dd, *J* = 17.6, 2.9; δ 2.38, dd, *J* = 17.6, 10.0) are further apart from each other than those of **35** (δ 2.85, dd, *J* = 17.0, 2.4; δ 2.42 dd, *J* = 17.0, 9.8). The optical rotation of synthetic 22-*epi*-berkelic acid (**36**), $[\alpha]_D^{22}$ − 107.0 (*c* = 0.43, MeOH), is very similar to that of berkelic acid indicating that the C-22 stereochemistry has a minor effect on the rotation as expected.

It still remained to assign the stereochemistry of **33–36** at C-22. Fráter reported the stereoselective α- alkylation of chiral β-hydroxy esters in excellent diastereoselectivity using chelation to control the stereochemistry of the alkylation.[25] Fráter's alkylation might allow us to use the hydroxy group stereochemistry introduced at C-21 in the Kiyooka aldol reaction to control the stereochemistry at the quaternary center C-22. We decided to explore this approach first in a model system. Addition of (*R*)-citronellal (**37**) to the lithium enolate of methyl butyrate (**38**) afforded **39** as a mixture of four isomers (see Scheme 8). Treatment of **39** with 2.2 equiv of LDA gave a mixture of chelated alkoxy enolates **40** and **41**, which was treated with MeI and HMPA as described by Fráter to give 16% of an inseparable 1:1 mixture of **42** and **43** containing a little **44** and **45**. This sequence typically proceeds with excellent relative stereocontrol, but in poor yield.[26] Therefore it isn't practical to use it with aldehyde **24** to prepare berkelic acid. However, it might be possible to prepare **42**–**45** with known stereochemistry and to assign the stereochemistry of **33**–**36** by comparison of the spectral data of hydroxy esters **33** and **34** with hydroxy esters **42**–**45**.

Reaction of (*R*)-citronellal (**37**) and **19** using *N*-Ts-(*S*)-valine ((*S*)-**32**) gave **42** (38%) and **44** (40%). A similar sequence with the mismatched *N*-Ts-(*R*)-valine ((*R*)-**32**) provided **43** (21%) and **45** (18%). Isomers **42** and **43** are less polar than **44** and **45**, so that these compounds can be easily separated. The stereochemistry can now be easily assigned. The compound produced by both Fráter's procedure, which controls the relative stereochemistry at C-2 and C-3, and Kiyooka's procedure using *N*-Ts-(*S*)-valine, which controls the absolute stereochemistry at C-3, must be **42**. Therefore, the other isomer produced from Kiyooka's procedure using *N*-Ts-(*S*)-valine must be **44**. The compound produced by both Fráter's procedure and Kiyooka's procedure using *N*-Ts-(*R*)-valine must be **43**. Therefore, the other isomer produced from Kiyooka's procedure using *N*-Ts-(*R*)-valine must be **45**.

The structure assignments of **42**–**45** were confirmed by Dess-Martin oxidation of **45** and **42** to keto ester **46** and of **43** and **44** to keto ester **47**, and by conversion of **42** and **44** to ketals **48** and **49**, respectively, whose stereochemistry was established by NOE studies (see Scheme 9).[27] At this point, the structure assignments of **42–45** were secure, although they are based on

the known selectivity of the Kiyooka aldol reaction for the *Si* face with *N*-Ts-(*S*)-valine, which has been confirmed in numerous examples.[23]

We then compared the spectral data of **42**–**45** with those of **33** and **34**. The subtle differences in the ^{1}H and ^{13}C NMR spectra of (21*R*,22*S*)-**33**, (2*S*,3*R*)-**42**, (2*R*,3*S*)-**43** on the one hand and (21*R*,22*R*)-**34**, (2*R*,3*R*)-**44**, and (2*S*,3*S*)-**45** on the other hand are identical in all 10 features that we can distinguish as shown in Tables S1 and S2 (see Supporting Information) suggesting that the stereochemistry of **33** and **34** can be assigned as shown by analogy to that of **42**–**45**. The chromatographic properties also support this assignment: **33**, **42**, and **43** are less polar than **34**, **44**, and **45**, respectively. Heathcock assigned the stereochemistry of aldol products by analysis of the ^{13}C NMR spectral data.[28] However, this approach was not extended to α,α-dialkyl aldol products and is not useful with our compounds.

Because berkelic acid was reported to show selective activity toward ovarian cancer OVCAR-3 with a GI_{50} of 91 nM,[1] we submitted synthetic berkelic acid (**35**) and 22-*epi*-berkelic acid (**36**) to the NCI human disease-oriented 60-cell line in vitro antitumor screening protocol. Unfortunately, neither of them showed significant activity against any cell lines at 10^{-5} M (see Supporting Information). The racemic model acid prepared by hydrolysis of **8a** inhibited lung cancer cell line HOP-92 and ovarian cancer IGROV1 very effectively at 10^{-5} M, but had little effect on other cell lines.

The proposed biosynthesis in Scheme 1 needs to be reconsidered in light of the revision of the stereochemistry of berkelic acid (**35**) at C-18 and C-19 and the assignment of the stereochemistry at C-22 and the absolute stereochemistry. The stereochemistry of berkelic acid (**35**) at C-9 is the same as that of pulvilloric acid (**6**). Berkelic acid co-occurs with spiciferone A (**2**), which makes it plausible that a spicifernin-like compound might be an intermediate in its biosynthesis. However, spicifernin (**3**) has the same stereochemistry as berkelic acid at C-22, but the opposite stereochemistry at C-18 and C-19. It is not immediately obvious how to relate the stereochemistry at C-22 of berkelic acid to that of the quaternary center of the congener spiciferone A (**2**). We therefore considered the biogenetic relationship between spiciferone A (**2**), spicifernin (**3**), and spiciferinone (**54**), which were isolated from same strain of *Cochliobolus spicifer*.[4]

The relative and absolute stereochemistry of spiciferone A (**2**) and spicifernin (**3**) have been assigned as shown.[4] From feeding studies with [1,2-^{13}C]- and [1-^{13}C,$^{2}H_3$]-acetate, Nakajima established that they are biosynthesized from acetate and *S*-adenosylmethionine and proposed that they are biosynthesized from **51** (see Scheme 10). Because CD_3 was incorporated only in the ethyl groups of spiciferone A (**2**) spicifernin (**3**), he suggested **51** was formed by a retro aldol reaction of a hydroxycyclodecatetraone.[4] However, deuterium washout from the starter acetate has been observed in the biosynthesis of monensin A and other natural products, so aldehyde **51** might be derived from the reduction of polyketide **50**.[29] An aldol reaction of **51** through pathway A should afford **52**, which could undergo dehydration to give spiciferone A (**2**). An aldol reaction of **51** through pathway B should afford **53**, which would undergo dehydration to give spiciferinone (**54**). Oxidative cleavage of **54** at the indicated bond will give aldehyde ester **55**. Hydrolysis of the pyran will give β-keto aldehyde **56** which can easily lose formic acid to give aldehyde **57**. This could also occur by oxidation of the aldehyde to an acid and decarboxylation of the β-keto acid. Oxidation of **57** will give spicifernin (**3**) and reduction will give the possible berkelic acid precursor **58**. The stereochemistry at the quaternary carbon center (C-22) was already set in **51**. However, the stereochemistry at C-18 and C-19 is set in the further elaboration of spiciferinone (**54**), which proceeds through easily epimerizable intermediates. Four isomers are possible, but equilibration can occur at either C-18 or C-19 before reduction or oxidation of aldehyde **57**. This scheme provides a simple biosynthetic pathway from the common intermediate **51** to spiciferone A (**2**), spicifernin (**3**), spiciferinone

(**54**) and berkelic acid precursor **58**. It is consistent with the known absolute stereochemistry at the quaternary carbon of the spiciferone A (**2**), spicifernin (**3**), and berkelic acid (**35**) and provides a simple explanation for the differing stereochemistry at C-18 and C-19 of spicifernin (**3**) and berkelic acid (**35**).

In conclusion, we have completed the first synthesis of (−)-berkelic acid (**35**), established the absolute stereochemistry, and assigned the stereochemistry at C-22. Although the aldol reaction to introduce the side chain is challenging, it proceeds effectively under Kiyooka's conditions using *N*-Ts-(*S*)-valine ((*S*)-**32**) to give only two of the four possible aldol products.

Experimental Section

Methyl (α*S*,β*R*,2*S*,3*S*,3'a*S*,4*S*,5'*R*)- and (α*R*,β*R*,2*S*,3*S*,3'a*S*,4*S*,5'*R*)- α,3-Dimethyl-α-ethyl- 3', 3'a,4,5,5',6'-hexahydro-β-hydroxy-3-methyl-5'-pentyl-8'-(2-propenyloxy)-9'-(2-propenyloxycarbonyl)-spiro[furan-2(3*H*),2'-[2*H*]pyrano[2,3,4-*de*][1]benzopyran]-4-butanoate (33 and 34)

To a solution of *N*-Ts-(*S*)-valine[22,23] (84 mg, 0.31 mmol) in dry CH_2Cl_2 (2 mL) was added BH_3•THF (0.31 mL, 1 M solution in THF, 0.31 mmol) by syringe over 3 min under N_2 at 0 °C. The solution was stirred for 30 min at 0 °C and additionally for 30 min at 25 °C. The solution was cooled −78 °C and a solution of aldehyde **24** (44.0 mg, 88.4 μ-mol) in CH_2Cl_2 (0.5 mL) was added dropwise over 3 min. After stirring for 5 min, silyl ketene acetal **19** (49 mg, 0.31 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise over 3 min. The reaction mixture was stirred for 4 h at −78 °C and quenched with 1 M aqueous HCl (4 mL). The mixture was allowed to warm to 25 °C and the aqueous layer was extracted with CH_2Cl_2 (4 × 5 mL). The combined organic layers were washed with saturated $NaHCO_3$ (2 × 10 mL), dried ($MgSO_4$) and concentrated to yield 71.6 mg of crude aldol product. Preparative TLC (4:1 hexanes/EtOAc, developed four times) gave 21.5 mg (40%) of **33** followed by 21.9 mg (40%) of **34**.

Data for **33**: $[\alpha]_D^{22}$ −95.0 (*c* 0.22, $CHCl_3$); ^{1}H NMR 6.23 (s, 1, H-4), 6.07–5.92 (m, 2), 5.40 (br d, 1, *J* = 17.1), 5.36 (br d, 1, *J* = 16.5), 5.25 (br d, 1, *J* = 9.8), 5.22 (br d, 1, *J* = 9.2), 4.86–4.72 (m, 3, H-15), 4.51 (d, 2, *J* = 4.9), 4.28 (dd, 1, *J* = 8.5, 8.5, H-26), 3.86–3.78 (m, 1, H-9), 3.77–3.69 (m, 1, H-21), 3.709 (s, 3, OMe), 3.65 (dd, 1, *J* = 8.5, 8.5, H-26), 2.75 (dd, 1, *J* = 17.1, 3.4, H-8), 2.60 (dd, 1, *J* = 17.1, 11.0, H-8), 2.27–2.20 (m, 1, H-19), 2.22 (d, 1, *J* = 6.1, OH), 2.14 (dd, 1, *J* = 12.2, 5.5, H-16), 1.96 (dd, 1, *J* = 12.2, 12.2, H-16), 1.80 (dq, 1, *J* = 13.4, 7.3), 1.71–1.60 (m, 3), 1.59–1.46 (m, 3), 1.45–1.24 (m, 6), 1.14 (s, 3, H-27), 1.03 (d, 3, *J* = 6.7, H-25), 0.90 (t, 3, *J* = 6.7), 0.87 (t, 3, *J* = 7.3); ^{13}C NMR 176.94, 165.66, 155.55, 149.09, 135.89, 132.96, 132.40, 118.17, 117.13, 114.65, 109.52, 108.52, 104.23, 76.22, 75.31, 73.87, 69.38, 68.11, 65.64, 51.79, 51.23, 49.04, 42.48, 36.32, 35.31, 34.55, 34.41, 31.79, 28.38, 25.14, 22.61, 16.75, 14.04, 11.87, 8.99; IR (neat) 3508, 2953, 2933, 2860, 1739, 1732, 1715; HRMS (EI) calcd for $C_{35}H_{50}O_9$(M^+) 614.3455, found 614.3451.

Data for **34**: $[\alpha]_D^{22}$ − 96.3 (*c* 0.22, $CHCl_3$); ^{1}H NMR 6.23 (s, 1, H-4), 6.07–5.92 (m, 2), 5.40 (br d, 1, *J* = 17.1), 5.36 (br d, 1, *J* = 16.5), 5.25 (br d, 1, *J* = 9.8), 5.22 (br d, 1, *J* = 9.2), 4.86–4.72 (m, 3, H-15), 4.51 (d, 2, *J* = 4.9), 4.31 (dd, 1, *J* = 8.5, 8.5, H-26), 3.86–3.77 (m, 1, H-9), 3.726 (s, 3, OMe), 3.74–3.68 (m, 1, H-21), 3.66 (dd, 1, *J* = 8.5, 8.5, H-26), 2.75 (dd, 1, *J* = 17.1, 3.4, H-8), 2.60 (dd, 1, *J* = 17.1, 11.0, H-8), 2.51 (d, 1, *J* = 7.3, OH), 2.30–2.21 (m, 1, H-19), 2.14 (dd, 1, *J* = 12.2, 4.9, H-16), 1.97 (dd, 1, *J* = 12.2, 12.2, H-16), 1.81–1.44 (m, 7), 1.43–1.17 (m, 6), 1.13 (s, 3, H-27), 1.04 (d, 3, *J* = 6.1, H-25), 0.90 (t, 3, *J* = 6.4), 0.85 (t, 3, *J* = 7.3); ^{13}C NMR 177.58, 165.63, 155.56, 149.10, 135.87, 132.96, 132.39, 118.11, 117.12, 114.64, 109.51, 108.43, 104.22, 75.49, 75.30, 74.00, 69.37, 68.11, 65.62, 51.86, 51.45, 49.07, 42.57, 36.31, 34.57, 34.49, 34.41, 31.78, 29.60, 25.13, 22.60, 16.94, 14.03, 11.87, 8.80; IR (neat) 3522, 2955, 2933, 2860, 1737, 1732, 1715; HRMS (EI) calcd for $C_{35}H_{50}O_9$ (M^+) 614.3455, found 614.3458.

Methyl (α*S*,2*S*,3*S*,3'a*S*,4*S*,5'*R*)-9'-Carboxy-α,3-dimethyl-α-ethyl-3',3'a,4,5,5',6'-hexahydro-8'-hydroxy-3-methyl-β-oxo-5'-pentyl-spiro[furan-2(3*H*),2'-[2*H*]pyrano[2,3,4-*de*][1]benzopyran]-4-butanoate (35, berkelic acid)

To a solution of $Pd(PPh_3)_4$ (6.1 mg, 5.3 μmol) and the above allyl ether and allyl ester prepared from **33** (16.2 mg, 26.4 μmol) in dry THF (2 mL) was added NEt_3 (148 μL, 1.06 mmol) and HCO_2H (40 μL, 1.06 mmol) under N_2 at 25 °C. The yellow solution was stirred at 25 °C for 15 h and quenched with saturated $NaHCO_3$ (5 mL). The aqueous layer was extracted with ether (4 × 5 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. Flash chromatography on silica gel (80:1:0 to 200:1:1 CH_2Cl_2/MeOH/AcOH) gave 11.1 mg (78%) of berkelic acid (**35**): $[\alpha]_D^{22}$ − 115.5 (*c* 0.55, MeOH); {lit.[1] $[\alpha]_D^{20}$ − 83.5 (*c* 0.0113, MeOH)}; ^{1}H NMR ($CDCl_3$, the residual peak of solvent is referenced as δ 7.24 rather than 7.27 to facilitate comparison with the literature data1) 11.82 (s, 1, OH), 11.13–10.92 (br, 1, OH), 6.42 (s, 1, H-4), 4.76 (dd, 1, *J* = 12.2, 5.4, H- 15), 4.44 (dd, 1, *J* = 8.5, 8.5, H-26), 3.84–3.76 (m, 1, H-9), 3.73 (s, 3, OMe), 3.59 (dd, 1, *J* = 8.5, 8.5, H-26), 2.85 (dd, 1, *J* = 17.0, 2.4, H-20), 2.78 (dd, 1, *J* = 17.7, 3.7, H-8), 2.60 (dd, 1, *J* = 17.7, 11.0, H-8), 2.54–2.45 (m, 1, H-19), 2.42 (dd, 1, *J* = 17.0, 9.8, H-20), 2.21 (dd, 1, *J* = 12.2, 5.4, H-16), 2.05 (dd, 1, *J* = 12.2, 12.2, H-16), 1.95 (dq, 1, *J* = 14.2, 7.3, H-23), 1.87 (dq, 1, *J* = 10.7, 6.8, H-18), 1.80 (dq, 1, *J* = 14.2, 7.3, H-23), 1.68–1.57 (m, 1), 1.58–1.43 (m, 2), 1.43–1.20 (m, 5), 1.32 (s, 3, H-27), 1.09 (d, 3, *J* = 6.8, H-25), 0.88 (t, 3, *J* = 6.6), 0.83 (t, 3, *J* = 7.6); ^{1}H NMR (CD_3OD, 500 MHz) 6.27 (s, 1, H-4), 4.72 (dd, 1, *J* = 12.2, 5.4, H-15), 4.30 (dd, 1, *J* = 8.5, 8.5, H-26), 3.84–3.77 (m, 1, H-9), 3.73 (s, 3, OMe), 3.50 (dd, 1, *J* = 8.5, 8.5, H-26), 2.88 (dd, 1, *J* = 17.6, 3.1, H-20), 2.78 (dd, 1, *J* = 17.3, 3.7, H-8), 2.70–2.62 (m, 1, H-19), 2.54 (dd, 1, *J* = 17.3, 11.2, H-8), 2.53 (dd, 1, *J* = 17.6, 10.5, H-20), 2.14 (dd, 1, *J* = 12.2, 5.4, H-16), 1.94 (dq, 1, *J* = 14.2, 7.3, H-23), 1.91 (dd, 1, *J* = 12.2, 12.2, H-16), 1.88–1.78 (m, 2, H-23, H-18), 1.63–1.48 (m, 3), 1.46–1.27 (m, 5), 1.32 (s, 3, H-27), 1.08 (d, 3, *J* = 6.3, H-25), 0.92 (t, 3, *J* = 6.8), 0.83 (t, 3, *J* = 7.6); ^{13}C NMR ($CDCl_3$) 206.0, 173.4, 170.5, 162.5, 149.8, 142.2, 112.2 (2 C), 110.5, 98.6, 75.2, 73.5, 67.2, 59.7, 52.5, 48.2, 41.6, 39.4, 36.2, 34.30, 34.29, 31.8, 27.9, 25.0, 22.6, 18.4, 14.0, 12.0, 8.7; ^{13}C NMR (CD_3OD, 400 MHz) 208.8, 174.8, 173.7, 163.4, 153.1, 142.3, 113.8, 110.7, 109.4, 101.1, 76.6, 74.2, 69.5, 61.0, 52.9, 42.6, 40.5, 37.4, 35.4, 35.0, 33.0, 28.9, 26.2, 23.7, 18.9, 14.4, 11.9, 9.0 (a peak near δ 49.2 is obscured by the solvent peak); IR (neat) 3238, 2957, 2933, 2860, 1713, 1694; HRMS (EI) calcd for $C_{29}H_{40}O_9(M^+)$ 532.2672, found 532.2659. The ^{1}H and ^{13}CNMR spectral data in both $CDCl_3$ and CD_3OD are identical to those of the natural product (see Tables S2–S5).[1]

Methyl (α*R*,2*S*,3*S*,3'a*S*,4*S*,5'*R*)-9'-Carboxy-α,3-dimethyl-α-ethyl-3',3'a,4,5,5',6'-hexahydro-8'-hydroxy-3-methyl-β-oxo-5'-pentyl-spiro[furan-2(3*H*),2'-[2*H*]pyrano[2,3,4-*de*][1]benzopyran]-4-butanoate (36, 22-*epi*-berkelic acid)

An identical reaction with the allyl ether and allyl ester prepared from **34** (14.0 mg, 22.8 μmol) afforded 8.7 mg (72%) of 22-*epi*-berkelic acid (**36**): $[\alpha]_D^{22}$ − 107.0 (*c* 0.43, MeOH); ^{1}H NMR ($CDCl_3$, the residual peak of solvent is referenced as δ 7.24 rather than 7.27 to facilitate comparison with the literature data1) 11.83 (s, 1, OH), 11.08–10.97 (br, 1, OH), 6.42 (s, 1, H-4), 4.77 (dd, 1, *J* = 12.2, 5.4, H-15), 4.45 (dd, 1, *J* = 8.5, 8.5, H-26), 3.84–3.76 (m, 1, H-9), 3.73 (s, 3, OMe), 3.59 (dd, 1, *J* = 8.5, 8.5, H-26), 2.90 (dd, 1, *J* = 17.6, 2.9, H-20), 2.79 (dd, 1, *J* = 17.6, 3.9, H-8), 2.60 (dd, 1, *J* = 17.6, 11.2, H-8), 2.54–2.44 (m, 1, H-19), 2.38 (dd, 1, *J* = 17.6, 10.0, H-20), 2.21 (dd, 1, *J* = 12.2, 5.4, H-16), 2.06 (dd, 1, *J* = 12.2, 12.2, H-16), 1.95 (dq, 1, *J* = 14.2, 7.3, H-23), 1.87 (dq, 1, *J* = 11.3, 6.8, H-18), 1.80 (dq, 1, *J* = 14.2, 7.3, H-23), 1.68–1.57 (m, 1), 1.58–1.43 (m, 2), 1.43–1.20 (m, 5), 1.33 (s, 3, H-27), 1.09 (d, 3, *J* = 6.8, H-25), 0.88 (t, 3, *J* = 6.6), 0.81 (t, 3, *J* = 7.3); ^{1}H NMR (CD_3OD, 500 MHz) 6.27 (s, 1, H-4), 4.73 (dd, 1, *J* = 12.2, 5.4, H-15), 4.30 (dd, 1, *J* = 8.5, 8.5, H-26), 3.84–3.77 (m, 1, H-9), 3.73 (s, 3, OMe), 3.50 (dd, 1, *J* = 8.5, 8.5, H-26), 2.92 (dd, 1, *J* = 17.6, 3.0, H-20), 2.79 (dd, 1, *J* = 17.3, 3.6, H-8), 2.72–2.62 (m, 1, H-19), 2.55 (dd, 1, *J* = 17.3, 10.3, H-8), 2.49 (dd, 1, *J* = 17.6, 10.5, H-20), 2.14 (dd, 1, *J* = 12.2, 5.4, H-16), 1.94 (dq, 1, *J* = 14.2, 7.3, H-23), 1.91 (dd, 1, *J* = 12.2,

12.2, H-16), 1.88–1.78 (m, 2, H-23, H-18), 1.64–1.49 (m, 3), 1.47–1.30 (m, 5), 1.33 (s, 3, H-27), 1.08 (d, 3, *J* = 6.3, H-25), 0.92 (t, 3, *J* = 6.8), 0.82 (t, 3, *J* = 7.3); ^{13}C NMR ($CDCl_3$) 206.0, 173.4 (tiny), 170.5, 162.5, 149.8, 142.2, 112.17, 112.15, 110.5, 98.6, 75.2, 73.5, 67.2, 59.7, 52.5, 48.2, 41.5, 39.3, 36.2, 34.31, 34.29, 31.8, 27.9, 25.0, 22.6, 18.3, 14.0, 12.0, 8.6; ^{13}C NMR (CD_3OD, 400 MHz) 208.7, 174.8, 173.6, 163.4, 153.1, 142.3, 113.8, 110.7, 109.4, 101.1, 76.6, 74.1, 69.5, 61.0, 52.9, 42.6, 40.5, 37.4, 35.4, 35.0, 33.0, 28.8, 26.2, 23.7, 18.8, 14.4, 11.9, 8.9 (a peak near δ 49.2 is obscured by the solvent peak); IR (neat) 3233, 2957, 2933, 2860, 1713, 1694; HRMS (EI) calcd for $C_{29}H_{40}O_9$ (M^+) 532.2672, found 532.2667.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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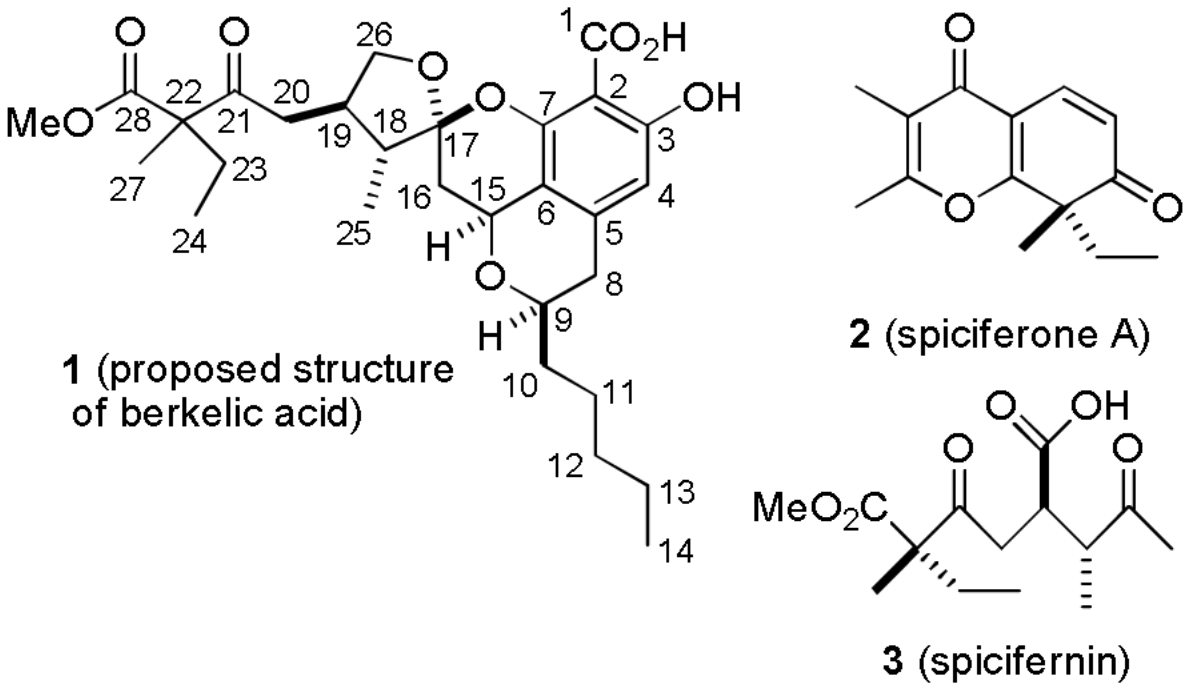


CHART 1.
Structures of Berkelic Acid (1), Spiciferone A (2), and Spicifernin (3)

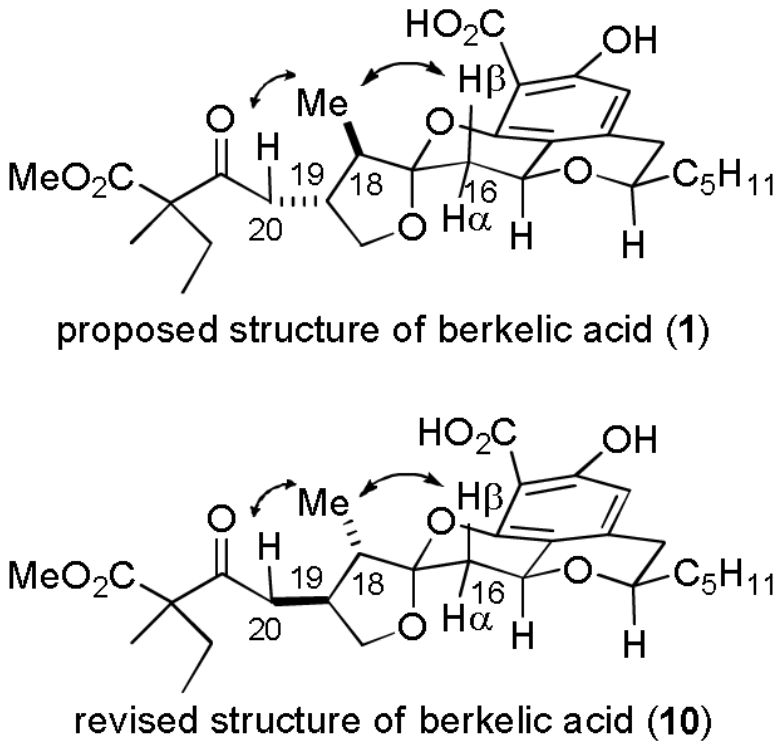
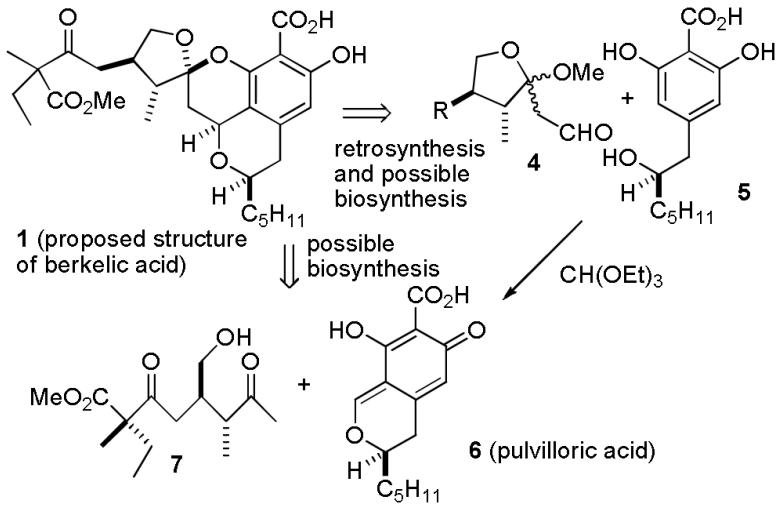
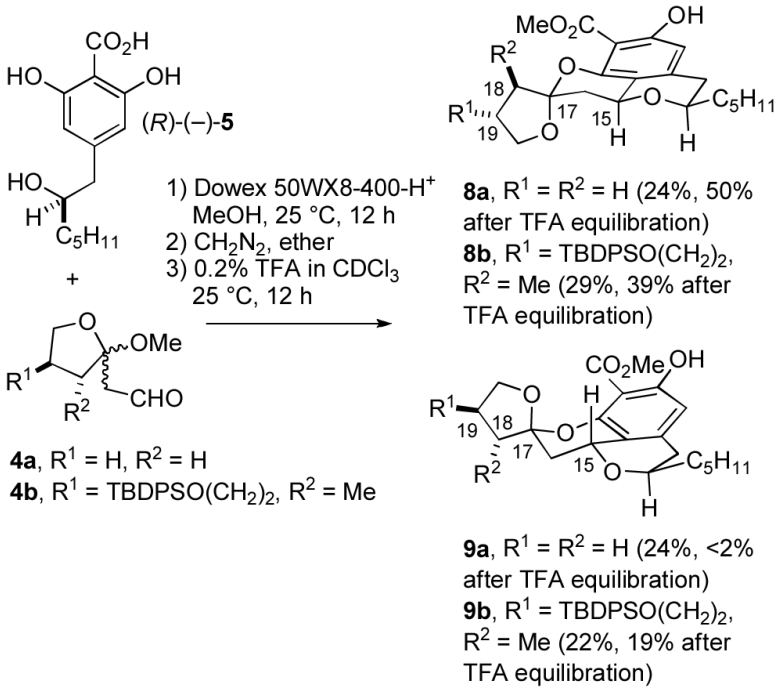


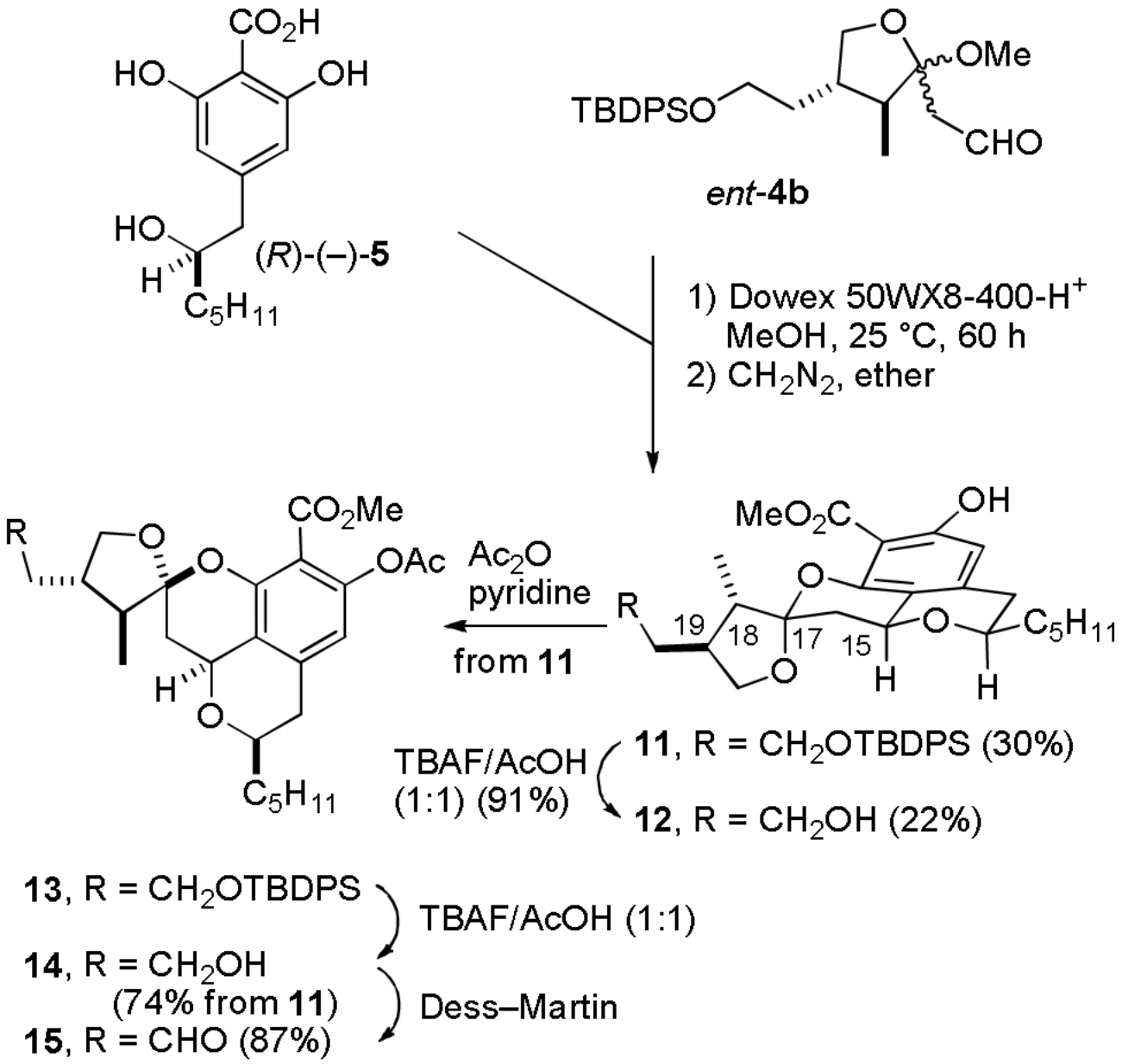
FIGURE 1.
Proposed and revised structures of berkelic acid showing NOE correlations to the C-25 methyl group.



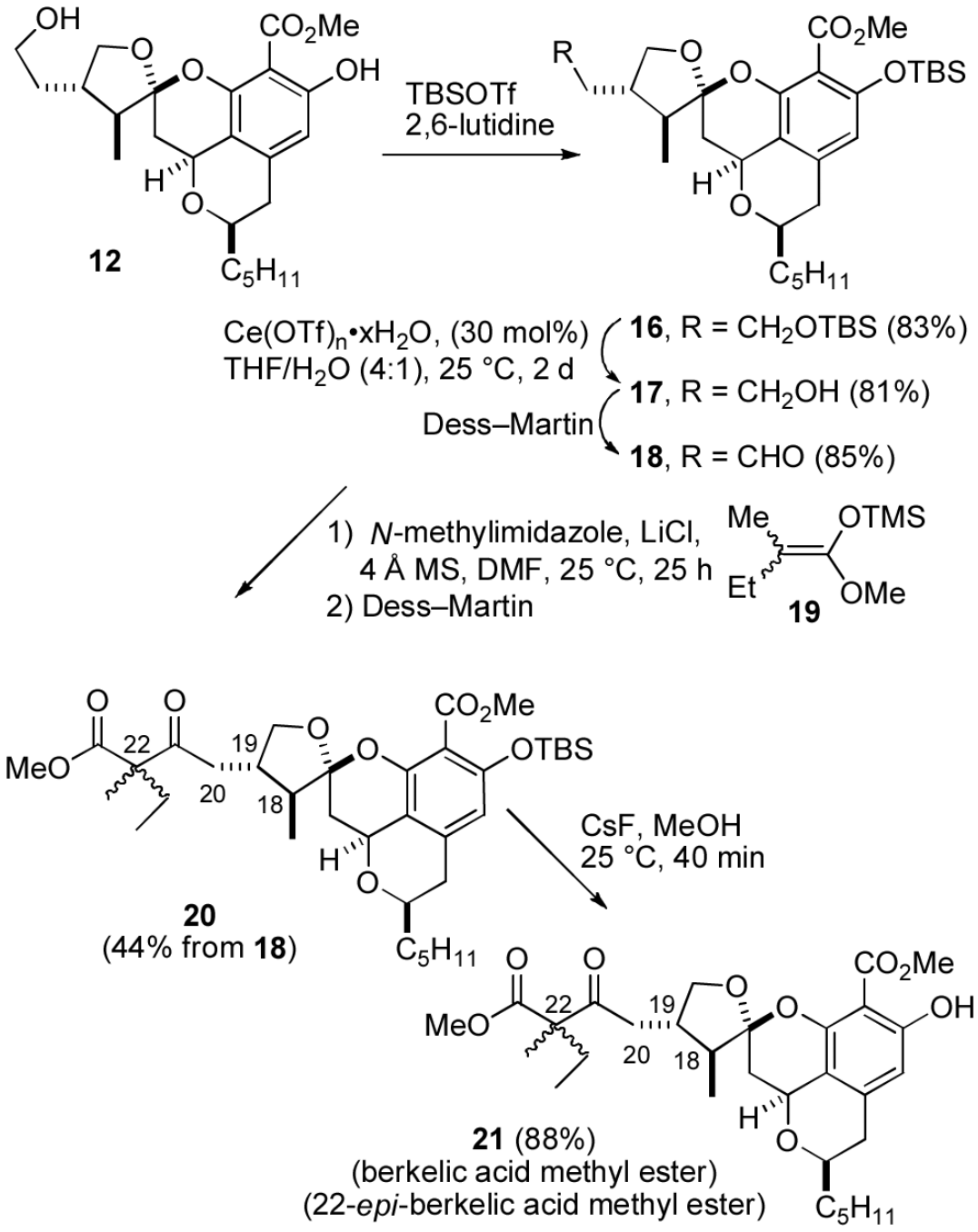
SCHEME 1.
Retrosynthesis and Possible Biosynthesis of (−)-Berkelic Acid (1)



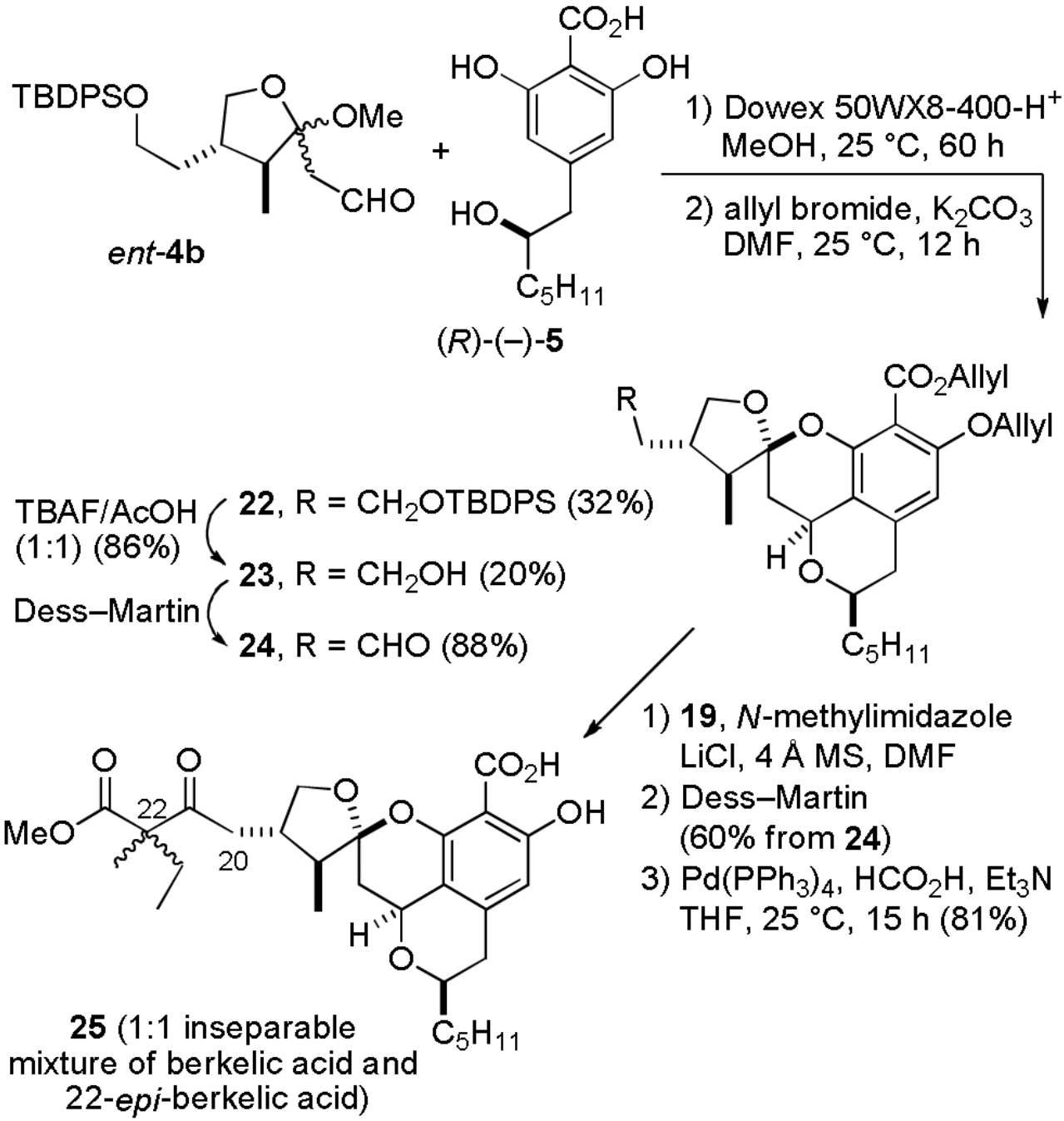
SCHEME 2.
Preparation of Tetracycles 8a and 8b



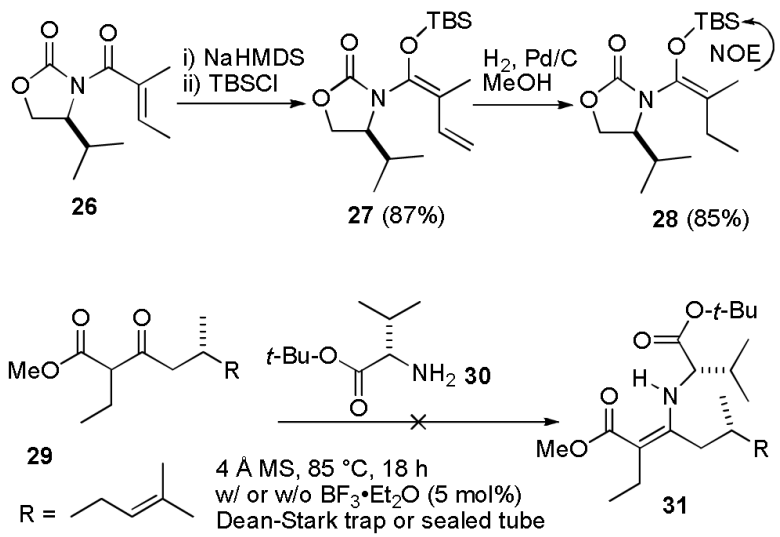
SCHEME 3.
Synthesis of Tetracyclic Acetoxy Aldehyde 15



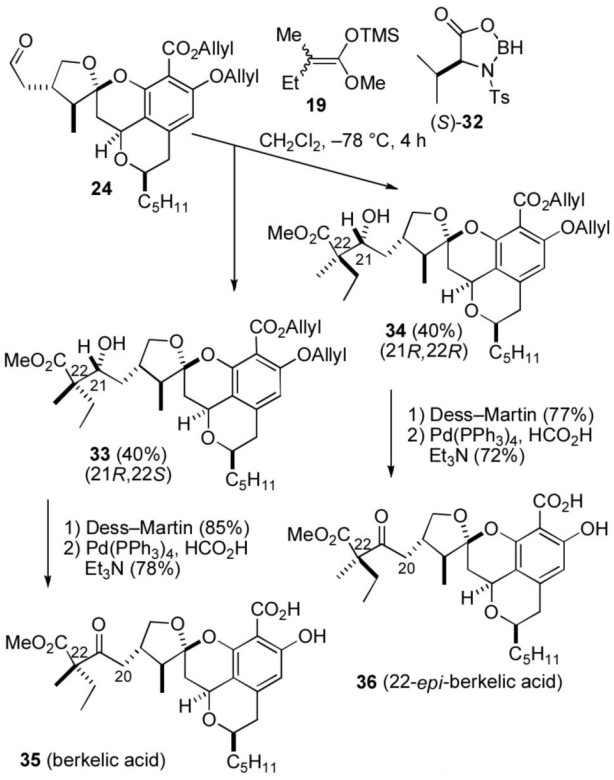
SCHEME 4.
Synthesis of a Mixture of Berkelic Acid Methyl Ester and the C-22 Epimer (21)



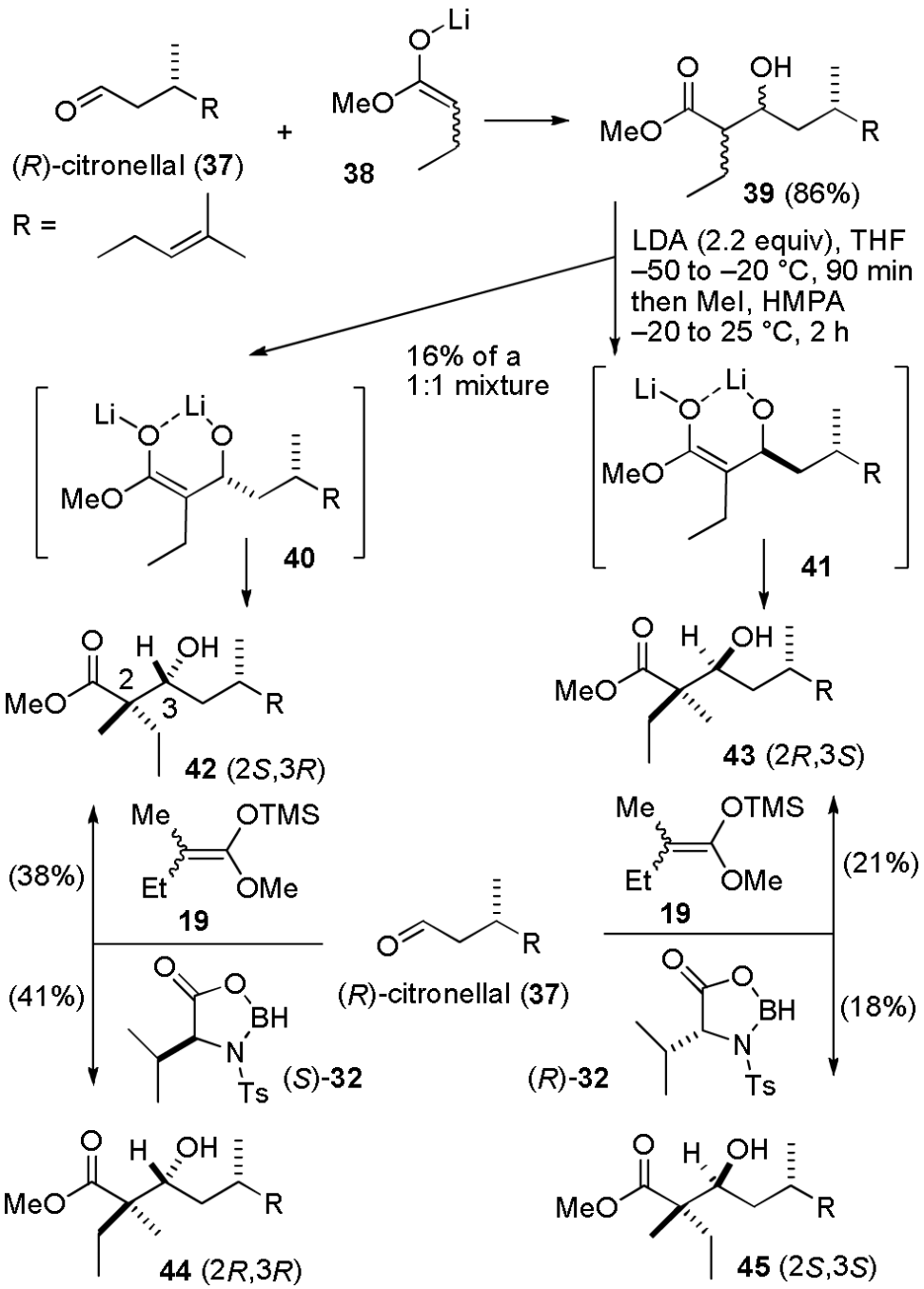
SCHEME 5.
Synthesis of a Mixture of Berkelic Acid and the C-22 Epimer (25)



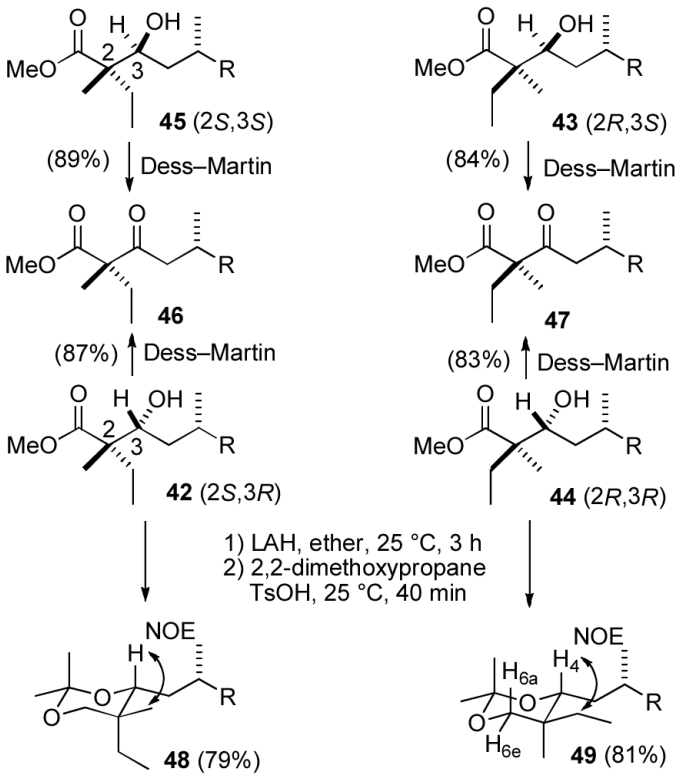
SCHEME 6.
Unsuccessful Aldol Reactions



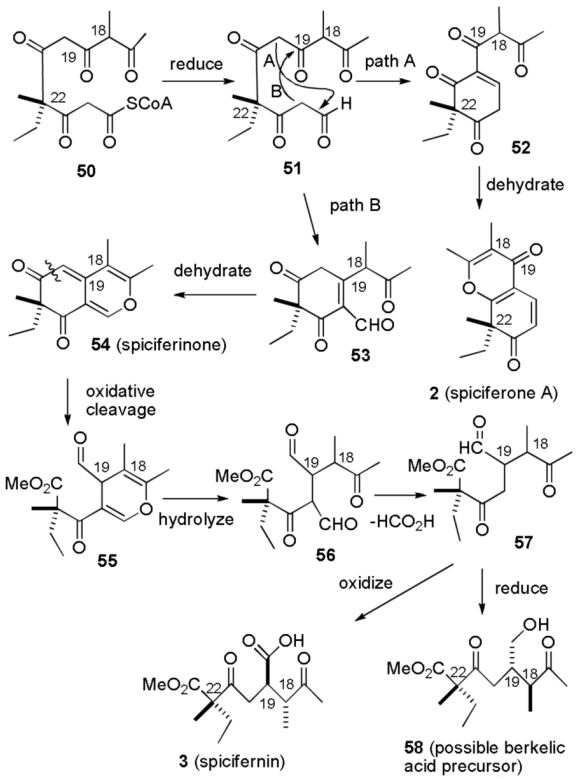
SCHEME 7.
Synthesis of Berkelic Acid (35) and 22-*epi*-Berkelic Acid (36)



SCHEME 8.
Synthesis of Aldol Adducts 42–45



SCHEME 9.
Structure Assignments of Aldol Adducts 42–45



SCHEME 10.

Proposed Biosynthesis of Spiciferone A (2), Spicifernin (3), Spiciferinone (54) and Possible Berkelic Acid Precursor (58)