Total synthesis of (±)-halichlorine, (±)-pinnaic acid, and (±)-tauropinnaic acid

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The related marine natural products halichlorine, pinnaic acid, and tauropinnaic acid have been synthesized. The described route provided access to all three compounds from a common, late-stage intermediate. The synthesis began with 1-pyrrolidino-1-cyclopentene from which an intermediate possessing the three contiguous stereocenters of the natural products was synthesized in just four steps. Olefin cross metathesis followed three contiguous stereocenters of the natural products was 1-cyclopentene from which an intermediate possessing the late-stage intermediate. The synthesis began with 1-pyrrolidino-dehydroquinolizidine ring system.

In 1996 Uemura and coworkers reported the isolation of halichlorine (1) (1), pinnaic acid (2) (2), and tauropinnaic acid (3) (2). These compounds were the first discovered and, so far, only members of a novel class of alkaloids characterized by a highly functionalized azaspiro[4.5]decane ring system. Halichlorine was isolated from the black marine sponge Halichondria okadai Kadota (1), whereas pinnaic acid and tauropinnaic acid were recovered from extracts of the Okinawan bivalve Pinna muricata (2). The structure of halichlorine was determined by using NMR methods (1), and the absolute configuration was elucidated by synthesis of a halichlorine degradation product (3). The structures of pinnaic and tauropinnaic acids were incompletely determined with NMR methods (2). Danishefsky's total synthesis of pinnaic acid (4, 5) resolved the initial conjecture (2) regarding the relative configuration at C14 and revealed the configuration at C17 of pinnaic and tauropinnaic acids. Both compounds are now known to have the same relative configuration as the equivalent substructure of halichlorine.

Halichlorine selectively inhibits the induced expression of vascular cell adhesion molecule 1 (1). Biological activity of this type is expected to be useful for the treatment of some inflammatory diseases (1, 6, 7). Both pinnaic acid and tauropinnaic acid exhibit inhibitory activity toward cytostolic phospholipase A2. This activity may also give these compounds anti-inflammatory properties (2, 8–10). Many research groups have reported research directed toward synthesis of these alkaloids (4, 5, 11–27). Danishefsky's research group has reported syntheses of both halichlorine (13, 14) and pinnaic acid (4, 5). Two formal pinnaic acid syntheses and one formal halichlorine synthesis, each intersecting Danishefsky's routes, have been reported (24, 26, 27).

Materials and Methods

Full experimental procedures and spectral data for all the compounds synthesized in this work (41 compounds, 39 procedures, and 37 pages) are given in Supporting Text, which is published as supporting information on the PNAS web site. Copies of the 1H NMR spectra of the synthetic natural products (±)-1, (±)-2, and (±)-3 are included in the supporting information.

Results and Discussion

Synthesis of a Common Intermediate. In this investigation the proposed synthesis plan for the core azaspiro[4.5]decane structure involved formation of the three contiguous stereocenters, C9, C13, and C14, by addition of a carbon nucleophile to an N-acylimmonium ion intermediate (5, Scheme 1). The cyclopentane ring alkyl substituent was expected to divert nucleophile attack to the other face of the ring during alkylation. Establishment of the C5 configuration was to result from reduction at that position (7 to 8, Scheme 1).

Acylimmonium ion precursor 13 was readily prepared from the known keto-alcohol 12, which is available as a separable 2:1 mixture of diastereomers by a published procedure (28). As shown in Scheme 2, condensation of this diastereomeric mixture of keto-alcohols with benzyl carbamate provided cis-fused bicyclic carbamates 13, epimeric at C14, in a 6:1 ratio. As expected, the major component of the mixture was the isomer with the

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methyl group on the convex face of the fused 5–5 bicyclic ring system. The same 6:1 diastereomer ratio was obtained regardless of whether the alcohol diastereomers were treated separately or as a mixture, demonstrating that epimerization occurs readily at the C13 position under the reaction conditions. Amines could also be converted directly into carbamates by activation with phenyl chloroformate followed by heating with benzyl carbamate. (Carbamates are also produced by treatment of amines with p-TsOH and benzyl carbamate, but only in modest yield.) The crystalline carbamates could not be separated by using flash chromatography or recrystallization, but separation was readily effected by using HPLC. Single-crystal x-ray analysis of the major isomer of carbamate confirmed the proposed structure (Fig. 1).

Addition of the mixture of carbamates to a solution of allyltrimethylsilane and TiCl₄ at −50°C provided, as the major product, alcohol (Scheme 3). (Attempted addition of more complicated nucleophiles was unsuccessful.) Alcohol is the expected product of addition to the less hindered face of the intermediate acylimmonium ion generated from the major isomer of carbamate (see structure 5 in Scheme 1). The by-product amino alcohol, resulting from loss of the carboxenzyo group, was converted into alcohol in ~60% overall yield by acylation with excess benzyl chloroformate, followed by saponification of the oxygen-bound carboxenzyo group. After acetylation of the primary hydroxy group, allyl derivative was subjected to olefin cross metathesis (29–32) with Nazarov ester (33) and Grubbs' second-generation metathesis catalyst (11) to efficiently produce enone, solely as the E isomer. Hydrogenation/hydrogenolysis of enone with hydrogen and a palladium catalyst produced piperidine as a single detectable isomer at the newly formed C5 stereocenter. The outcome of this reaction was expected based on a very similar reaction reported by Arimoto and coworkers (12, 24).

With the core of the natural products prepared, elaboration of both the C5 and the C13 side chains was required. Construction of the C13 side chain was pursued first as that approach allowed for the synthesis of both pinnaic acid and halichlorine from the same intermediate. Formation of the C15=C16 double bond with Weinreb's phosphonate (28) (11) (Scheme 4 Inset) required oxidation of the C15 primary alcohol to the corresponding aldehyde. However, it is well known from published work in similar systems that protection of the proximal nitrogen is difficult because of its extremely hindered steric environment (4, 5, 13, 14). We decided to use a β-lactam group for simultaneous protection for the nitrogen atom and the C5 side chain during C13 side-chain elaboration (Scheme 4). To this end, amino ester was converted to the corresponding amino acid by treatment with trifluoroacetic acid. β-lactam formation was effected in good yield by using modified Mukaiyama reagent (34). Acetate cleavage pro-

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*Danishefsky and coworkers found, after some experimentation, that the trifluoroacetate group was suitable for this application (4, 5).*
Oxidation of alcohol 23 with tetrapropylammonium per-ru-thenate and N-methylmorpholine N-oxide (35) afforded the aldehyde 25 in excellent yield, but an attempted Horner–Wadsworth–Emmons reaction with Weinreb’s phosphonate (28) (11) produced dienone 26 in low yields (Scheme 4). The best conditions found involved the Masamune–Roush procedure (36), which consistently gave the desired dienone 26 in ~25% yield. Other workers have reported similarly low yields from reaction of phosphonate 28 with a pinnaic acid precursor (5, 24). Our experiments suggested that the phosphonate reagent was the source of the problem, and an alternative coupling reagent was sought. Carboxylic acid 27, an intermediate in Weinreb’s phosphonate synthesis, was transformed into phosphorane 29 by reaction of the pivaloyl-mixed anhydride with methylene-triphosphonate (28) (Scheme 4). When heated with aldehyde 25 in methanol, phosphorane 29 reacted efficiently to provide dienone 26 in good yield.

Reduction of the ketone group, to form the fifth and final stereocenter of the natural products, was investigated by using a number of reagents (Scheme 5). Most notably, use of Luche’s (38) conditions resulted in a 5:2 mixture favoring the desired diastereomer (30) (the assignment of configuration was only known with certainty after completion of the syntheses), and use of (S)-Alpine hydride (39) favored formation of the undesired isomer (31) by a ratio of ~2:1. Protection of the newly formed hydroxyl group as a triethylsilyl ether completed the C13 side chain. In an asymmetric synthesis the use of a chiral reducing agent might be effective to force reagent control in the C17 ketone reduction.

Compounds 32 and 33 were transformed to pinnaic acid and 17-epi-pinnaic acid, respectively, by using the same transformations. [For clarity only the conversion of lactam 32 to (±)-pinnaic acid is described at this point. See the supporting information for details of the conversion in the 17-epi series.] Having served its purpose as dual protection for the amine and for the C5 side chain, it was next necessary to cleave the β-lactam. Amino aldehyde 35 (Scheme 6) was expected to be stable toward intermolecular nitrogen–aldehyde condensation reactions because of the extreme steric hindrance about the nitrogen atom, the same property that makes protection of this atom difficult. Reduction of lactam 32 with disobutylaluminum hydride provided some aldehyde but also alcohol and unreacted lactam. Reduction to the amino alcohol by using lithium triethylbоро-
hydride followed by oxidation using tetrapropylammonium per-
 ruthenate/N-methylmorpholine N-oxide (35) provided the
 amino aldehyde 35 in acceptable yield. The most effective
 reagent for direct reduction to the aldehyde was “Red-Al” (40),
a pyrrolidine-modified Red-Al (sodium bis(2-methoxyethoxy) 
 aluminum hydride). Use of this reagent allowed direct access to
 amino aldehyde 35 by the intermediacy of enamine 34, which
 readily decomposed to aldehyde 35 on contact with silica gel
 (Scheme 6).

 **Pinnaic Acid.** Horner–Wadsworth–Emmons reaction of aldehyde
 35, using triethyl 2-phosphonopropionate under the Masamune–
 Roush conditions (36), provided the desired trisubstituted diene
 36 in acceptable yield and high E/Z selectivity (Scheme 7). Forma-
 tion of the trisubstituted double bond completed construc-
 tion of the carbon skeleton of pinnaic acid; completion of
 the synthesis required only removal of the protecting groups.
 The silicon protecting groups were removed first because of the
 anticipated difficulties associated with handling free amino
 acids. Both silicon protecting groups were removed on treatment
 with tetrabutylammonium fluoride in tetrahydrofuran (THF),
 producing amino diol 37. Ester cleavage was best effected by
 using NaOH in aqueous methanol. The product of this reaction
 was dissolved in aqueous pH 7.00 buffer, extracted out by using
 1-butanol, and purified by reverse-phase HPLC. A deuteri-
 omethanol solution for NMR analysis was treated with NaOH,
 presumably forming the sodium carboxylate salt 38. This sample
 had the same NMR spectrum as observed for the initial hydro-
 lysis product. Another sample of the presumed zwitterion 2
 was treated with trifluoroacetic acid, presumably producing
 the ammonium trifluoroacetate salt 39. Comparison of the 1H NMR
 data for these samples indicated that the spectra have a very
 different appearance, depending on the state of protonation
 of pinnaic acid. Recently, NMR data for the sodium carboxylate
 and trifluoroacetate salt of pinnaic acid have been reported (24).
 By comparing our 1H NMR data for presumed zwitterion 2,
 conjugate acid 39, and conjugate base 38 with the spectra
 reported for the natural product, we conclude that pinnaic acid
 originally isolated by Uemura and coworkers (2, 24) was most
 likely the zwitterion, carboxylate, or a mixture of these two
 forms.

 **Tauropinnaic Acid.** The sodium salt of pinnaic acid (38) was
coupled with taurine under standard conditions (Scheme 8).
 After reverse-phase HPLC purification, a compound was iso-
lated that had identical 1H NMR data to those available (2) for
 tauropinnaic acid.

 **Halichlorine.** Conversion of amino aldehyde 35 into halichlorine
 required the formation of two new rings. The method of
 Semmelhack et al. (41) was used to synthesize an alkene poss-
sessing an appropriate functional handle for ring construction.
 Treatment of trimethyl phosphonoacrylate with lithium thiophenoxide
 followed by amino aldehyde 35 provided a mixture of
 Z- and E-thioethers 40 and 41 (Scheme 9). As observed by
 Semmelhack et al. (41) treatment of the E-thioether 41 with
 excess thiophenoxide allowed equilibration to a mixture con-
taining mostly the Z-isomer 40. Several methods, each involving
 activation of the sulfur atom, were considered for the conversion
 of the thioether 40 into dehydroquinolizidine 43. However,
simply heating a basic thiophenoxide solution of thioether 40 or
 the mixture of thiethers 40 and 41 resulted in formation of the
 dehydroquinolizidine 43 directly. The cyclization occurs,
 presumably, by addition/elimination reactions of thiophenoxide,
 ultimately allowing the nitrogen atom to add to the unsaturated
 ester of intermediate 42. In principle, the alkene synthesis
 and ring closure could be effected in one step by using catalytic
 thiophenoxide. However, the best conditions so far developed
 use the two-step procedure. The silicon protecting groups were
 cleaved with tetrabutylammonium fluoride, and the resulting

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*The moderate yield of this reaction may be due, in part, to methyl ester cleavage. Thiolates
have been deliberately used to effect ester cleavage (42, 43).*
nixture. The 1H NMR spectrum of synthetic (cyclization onto the C17 alcohol was not detected in the product THF, 0°C, 3 h; (d) NaOH, MeOH, H2O, 55°C, 2 h, then rt, overnight; (e) N-(3-methylaminopropyl)-N'-ethylcarbodiimide hydrochloride, N-dimethylaminopyridine, N-dimethylaminopyridine-HCl, CHCl3, THF, reflux, 10 h.

diol ester 44 was saponified to give the sodium salt. (±)-Halichlorine (1) was obtained in modest yield by subjecting the presumed sodium salt to Keck’s macro lactonization conditions (44). The isomeric lactone that would have resulted from cyclization onto the C17 alcohol was not detected in the product mixture. The 1H NMR spectrum of synthetic (±)-halichlorine was identical with that reported for the natural product (1). Natural halichlorine was isolated as a crystalline solid, but no crystal structure has been reported. Slow recrystallization of racemic halichlorine (1) from MeOH/H2O afforded x-ray quality crystals, and Fig. 3 shows the crystal structure of (±)-halichlorine. The crystal structure demonstrates the cis ring fusion in the dehydroquinolizidine system, which was predicted by Trauner et al. (46).

Conclusion

In summary, racemic amino aldehyde 35 has been synthesized in 15 linear steps beginning with pyrrolidinocyclopentene. (±)-Pinnac acid (2), (±)-tauropinnac acid (3), and (±)-halichlorine (1) have been synthesized in three, four, and five steps, respectively, from common intermediate 35. Because they were all prepared from aldehyde 35, which contains all the stereo centers, the x-ray structure of halichlorine provides a direct, unambiguous correlation of the stereoremiscence of the three natural products.

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