Natural Products

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Total Synthesis of Peribysin E Necessitates Revision of the Assignment of its Absolute Configuration**

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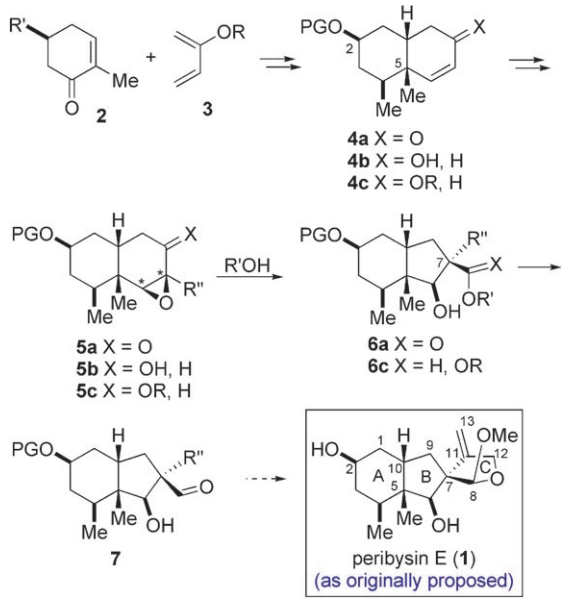
Several approaches have been followed in screening for small molecule natural products (SMNPs) which might provide leads to drug candidates in oncology.[1] One approach involves SMNPs which attack the diseased cell itself by targeting some part of the enzymatic or receptor machinery[2] necessary for upkeep of its neoplasticity. Such agents are broadly described as cytotoxic or antiproliferative. In another approach, the SMNP targets a strategic element of the support and maintenance systems required for tumor metastasis (for example, adhesion,[3] migration,[4] and angiogenesis[5]). Such an agent may be less toxic than an antiproliferative SMNP, although issues of selectivity of action must still be addressed. The most promising setting for the successful application of antimetastatic drugs would be one in which the primary tumor had been resected by surgery, radiation, or chemotherapeutic means (presumably through the use of cytotoxic drugs). The antimetastatic agent would be evaluated for its effect on halting or retarding the progression of the disease.

Thus, we took note of a series of disclosures by the research group of Yamada in which naturally occurring cell-adhesion inhibitors were described.[6,7] More specifically, we became interested in peribysin E, an adhesion inhibitor isolated from *Periconia byssoides* OUPS-N133, originally separated from the sea hare, *Aplysia kurodai*.[8] It was claimed that peribysin E is a stronger inhibitor than herbimycin.[6]

Accordingly, we initiated a program directed at the total synthesis of peribysin E, thereby hoping to provide chemical support for the exploration of the usefulness adhesion inhibitory natural products, and strategic congeners thereof, in the treatment of cancer. Moreover, our plan for the total synthesis of the natural product (described below) would confront mechanistic and stereochemical issues which are of considerable interest beyond this particular investigation.

We set as a late target, an intermediate aldehyde of the type **7**, confident that its conversion into peribysin E could be accomplished (Scheme 1). The organizing element of our plan was that rearrangement, with ring contraction, of an epoxide of the type **5** would result in formation of a five-membered ring containing the C7 quaternary center, which is present in peribysin E (see **6a** and **6c**, Scheme 1). The relationship of the



Scheme 1. Synthetic strategy toward peribysin E. PG = protecting group.

emerging C7 center to other resident stereogenic loci would be controlled by inversion of the configuration of the appropriately β-configured epoxide (see asterisks in **5**, Scheme 1) in the rearrangement step. The substrates **5a–c** specify alternative oxidation levels and protection states in the "migrating carbon atom" leading to products of the type **6**. In the case of the keto-type precursor **5a**, the desired rearrangement affords the future C8 center as an unspecified acyl derivative **6a**, which requires overall a two-electron reduction to eventually arrive at **7**. In the case of the precursors **5b** or **5c**, at the alcohol oxidation level, rearrangement with ring contraction would furnish C8, already at the

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aldehyde level. The free alcohol **5b** would be expected to progress directly to aldehyde **7**. In the case of protected alcohol **5c**, depending on the characteristics of the protecting group and the reaction conditions, rearrangement could lead directly to **7** or to an acetal, generalized as **6c**, which would then be converted into aldehyde **7**.

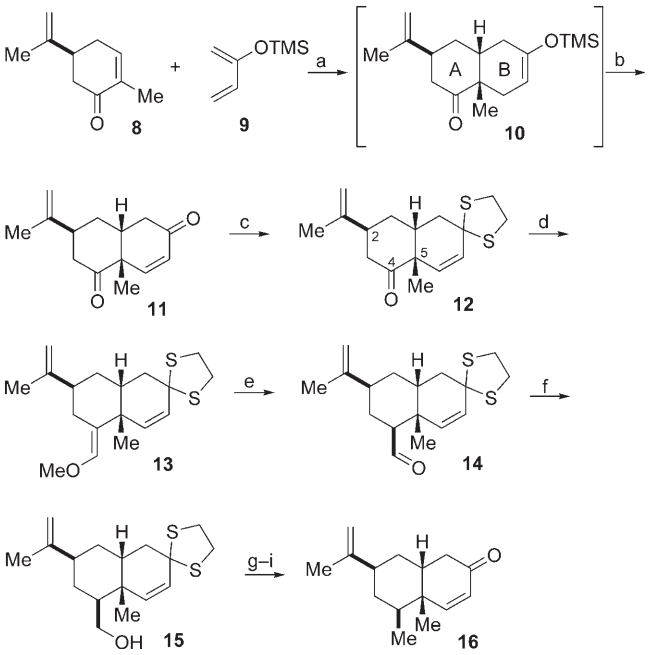
Study of the literature revealed surprisingly few encouraging examples of ring-contracting rearrangements of fused cyclohexene epoxides bearing an internal oxygen nucleophile of the type shown in **5**→**7**, or **5**→**6**→**7**. In fact, one could foresee other and better precedented reaction modalities for an epoxide of type **5**.[9] Indeed, in the course of events described below, several competitive pathways were encountered. To counter these ominous precedents, we hoped to set up a system where the required ring contraction would be favored. Fortunately, with forethought and persistence, the central plan prevailed, as potentially competitive pathways were suppressed with high margins of selectivity.[10] A total synthesis of the correctly configured peribysin E is described herein. As will be seen, our total synthesis program necessitated revision of the assignment of the absolute configuration of peribysin E.

The starting material initially employed for our synthesis was (*S*)-carvone (**2**, R′ = isopropenyl).[11] At a suitable time, the isopropenyl group at the C2-position would lend itself to conversion into the hydroxy function required for peribysin E, with strict preservation of configurational integrity. It was further expected that in the earlier stages, the isopropenyl group (R′) would block the β face of the dienophile and prompt cycloaddition from the α face. In this way, the *cis* relationship of the methyl group (corresponding to the C5-position of peribysin E) with the hydroxy group at C2, would be secured.

The synthesis began with the cycloaddition of (*S*)-carvone (**8**) with diene **9** under Lewis acid catalysis, thereby affording adduct **10**[12] (Scheme 2). The latter was well disposed to undergo Saegusa oxidation to give compound **11**. Selective thioketalization of the enone afforded **12**.[13] In this way, the Diels–Alder reaction was used to establish the required relative stereochemistry between the C2- and C5-positions, while furnishing a conjugated system with useful functional groups on which to build the properly configured spiro BC moiety.

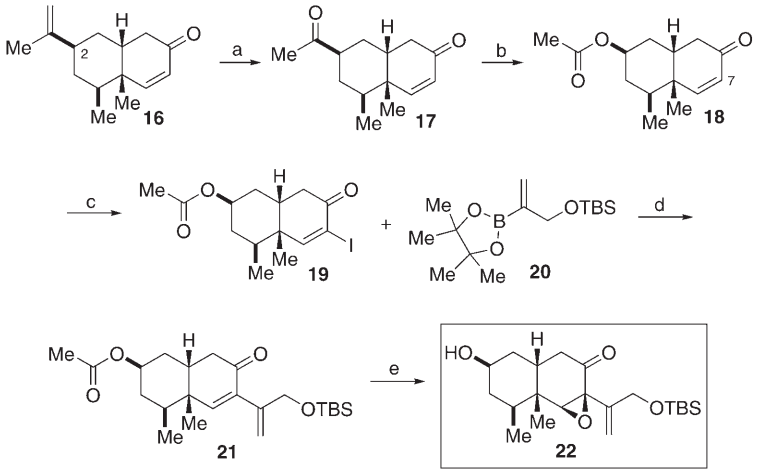
We next considered the introduction of the secondary methyl group, which corresponds to the C4 center of peribysin E. Wittig–Levine methoxymethenylation[14] of **12** gave rise to a product which, upon hydrolysis, emerged as aldehyde **14**. Reduction to **15** was followed by mesylation and a second hydride reduction.[15] Deprotection of the dithiane provided **16**.

Attention was now directed to the C2-position (peribysin numbering). The isopropenyl group was converted by a concurrent Johnson–Lemieux oxidation[16] into the corresponding ketone **17** (Scheme 3). Following Baeyer–Villiger oxidation, the desired relative configuration of the secondary acetate at C2 for our total synthesis was in hand



Scheme 2. Reagents and conditions: a) $EtAlCl_2$, toluene, 0 °C to RT, 4 h; b) $Pd(OAc)_2$, CH_3CN, RT, 4 h, 63% over 2 steps, 19:1 *cis*/*trans*; c) 1,2-ethanedithiol, MeOH, $BF_3{\cdot}OEt_2$, 0 °C, 30 h, 83%; d) $Ph_3PCH_2OCH_3Cl$, $KN(SiMe_3)_2$, THF, −30 °C to 0 °C to RT, 24 h; e) 4 N aq HCl, MeOH, THF, 0 °C to RT, 36 h, 89% over 2 steps, 13:1 β/α; f) $NaBH_4$, MeOH, THF, 0 °C to RT, 2 h, 90%; g) MsCl, Et_3N, CH_2Cl_2, 0 °C to RT, 1.5 h; h) $LiBHEt_3$, THF, 0 °C to RT, 24 h, 71% over 2 steps; i) $(CF_3CO_2)_2IPh$, MeOH, H_2O, CH_2Cl_2, RT, 15 min, 87%. TMS = trimethylsilyl.

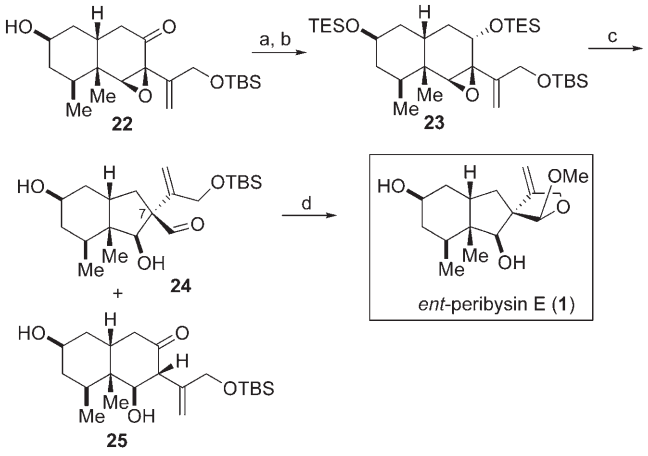
(**18**, Scheme 3). Our next goal was the introduction of a function which would enable cross-coupling α to the enone carbonyl group; that is, at the C7-position in the anticipated peribysin E. In the event, an iodide group was introduced, which afforded α-iodoenone **19**. Fortunately, cross-coupling



Scheme 3. Reagents and conditions: a) OsO_4, H_2O, $NaIO_4$, 2,6-lutidine, dioxane, RT, 4 h, 85%; b) MCPBA, CH_2Cl_2, 0 °C to RT, 24 h, 45% (80% based on recovered starting material); c) $TMSN_3$, I_2, pyridine, CH_2Cl_2, 71% (100% based on recovered starting material); d) $[Pd(PhCN)_2Cl_2]$, THF/H_2O, Ag_2O, Ph_3As, RT, 4 h, 89%; e) H_2O_2, NaOH, MeOH, 0 °C, 48 h, 85%. TBS = *tert*-butyldimethylsilyl, MCPBA = *meta*-chloroperoxybenzoic acid

of **19** with vinyl boronate **20**[17] gave rise to **21**. The key stereodefining step of the synthesis was accomplished upon nucleophilic epoxidation of **21**.[18] This reaction, which presumably commenced with axial attack of the hydrogen peroxide anion at the β carbon atom of the enone, produced compound **22** stereospecifically.

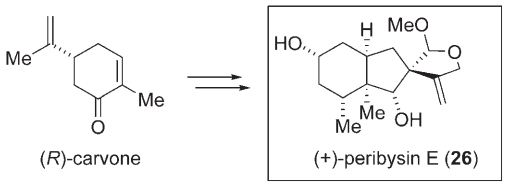
We found that the best route to peribysin E from compound **22** began by treatment with sodium borohydride (Scheme 4).[19] Triethylsilylation of the major alcohol provided silyl ether **23**. Remarkably, treatment of **23** with titanium tetrachloride afforded, as the principal product (in 50% yield), aldehyde **24** with the required relative configuration at the quaternary carbon atom (C7 of peribysin E).[20] Also produced in this reaction was a 5% yield of the β-hydroxy ketone **25**.[21,22]



Scheme 4. Reagents and conditions: a) $NaBH_4$, MeOH, THF, 0°C, 45 min, 91%, 7:1 α/β; b) TESCl, imidazole, DMF, RT, 12 h, 93%; c) $TiCl_4$, CH_2Cl_2, −78°C, 5 min, 50%, 10:1 (**24**/**25**); d) HCl, MeOH, 0°C, 1 h, 80%. DMF = dimethylformamide, TES = triethylsilyl.

Treatment of **24** with HCl/methanol indeed afforded the target system, peribysin E. The ^1H and ^{13}C NMR spectra of the synthetic peribysin E corresponded to those provided for naturally derived peribysin E (although the spectra of the synthetic material were cleaner).[23] We noted, however, a huge discrepancy in the measurements of the optical rotation: for the synthetic homogeneous product **1**, $[\alpha]_D^{25} = -52.17$ ($c = 0.11$, ethanol), whereas for natural peribysin E, $[\alpha]_D^{22} = -262.2$ ($c = 0.11$, ethanol). Although one might seek some level of encouragement from the fact that the reported and observed values were both negative, the numerical discrepancy was so large as to raise serious concerns on the issue of identity. The situation was clarified by preparing the diacetate of synthetic peribysin E. Here, a rotation of $[\alpha]_D^{25} = -34.78$ ($c = 0.069$, EtOH) was found for synthetic peribysin E diacetate, compared with $[\alpha]_D^{25} = +35.00$ ($c = 0.069$, EtOH) reported for the naturally derived peribysin E diacetate. We could only reason that the original assignment of the absolute configuration for peribysin E was incorrect.

To clear up any confusion, we then repeated all the steps of the total synthesis starting with (*R*)-carvone (Scheme 5). Not surprisingly, we reached peribysin E with the absolute configuration shown in **26**. Indeed, the value for the optical rotation of the synthetic peribysin E diacetate derived from (*R*)-carvone was $[\alpha]_D^{24} = +37.49$ ($c = 0.069$, EtOH). Given the validity of the assignments of the absolute configurations of the carvone enantiomers,[11] and fully confident of the stereochemical logic of our syntheses, we conclude that peribysin E is properly represented as **26**, and that our original target, stereostructure **1**, in fact corresponds to *ent*-peribysin E.



Scheme 5. Synthesis of (+)-peribysin E (**26**).

Our first suspicions that the initial total synthesis had produced *ent*-peribysin E actually arose from cell-adhesion measurements. The initial product **1** was essentially inactive in the HUVEC assay. Puzzled by this discrepancy,[6,7] we speculated that perhaps the absolute configuration of peribysin E had been misassigned. At that point, we conducted the diacetate comparison discussed above. With compounds **1** and **26** now available by total synthesis, they could each be subjected to the HUVEC assay (Figure 1). In the adhesion assay of human leukemia HL60 cells to HUVEC, a dose-dependant effect was observed for peribysin E (**26**), first being detectable at 4 μM and reaching a maximum at 100 μM. At concentrations above 100 μM, HUVEC began to detach from the bottom of the well in response to the drug treatment, thus rendering adhesive evaluation specific to HL60 unfeasible. By comparison, *ent*-peribysin E (**1**) only revealed minor

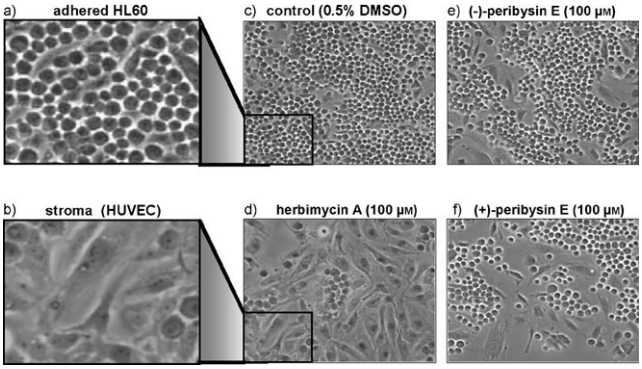


Figure 1. Biological evaluation of (+)-peribysin (**26**) and (−)-peribysin (*ent*-peribysin, **1**) at 100 μM. Adhesion of the leukemic cell line HL60 to the primary human-umbilical-vein endothelial cells (HUVEC). a) Magnifications of adhered HL60 are given, which depict HL60 as dark cells with white halos. b) The stroma are shown without attached leukemic cells, thus demonstrating the classic spread-out morphology. Examples of the HL60 adhesion assay are presented: c) control experiment exhibiting HL60 adhesion, d) lack of HL60 adhesion after treatment with herbimycin A at 100 μM. The enantiomers of peribysin E are depicted at 100 μM: e) the nonnatural enantiomer **1** and f) the naturally occurring (+)-peribysin E (**26**) demonstrating partial blockage of adhesion.

influences on HL60 adhesion at concentrations above 50 μM. Thus, the antiadhesive property of peribysin E is enantiospecific.

Although this study had accomplished its immediate goal, that is, the total synthesis of peribysin E, and had, in fact, required the revision of the assignment of absolute configuration from **1** to **26**, several questions have been raised regarding mechanism and stereoelectronic details, which go far beyond the confines of the peribysin area. Several fascinating issues in this regard are being revisited and will be reported in due course.

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