ASYMMETRIC SYNTHESIS OF RHOPALOIC ACID A ANALOGUES AND THEIR BIOLOGICAL PROPERTIES

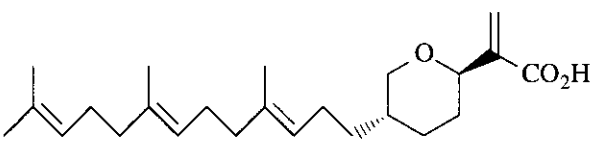
Hiroko Nishitani, Asami Sasaoka, Munetaka Tokumasu, and Katsuo Ohkata*

Department of Chemistry, Faculty of Science, Hiroshima University,
1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan

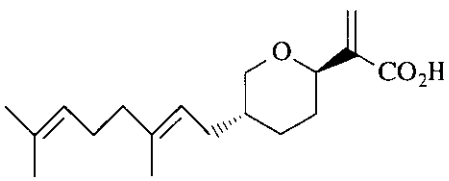
Abstract - Some of rhopaloic acid A analogues were synthesized and their bioactivity was investigated on the basis of the inhibition of gastrulation of sea urchin embryos.

The interesting biological activity of rhopaloic acid A [(+)-**1**], a potent cytotoxic agent, which was isolated from a marine sponge, *Rhopaloeides* sp.,[1] may be attributed to the structurally unique feature of having a hydrophilic tetrahydropyranylacrylic acid moiety connected to a hydrophobic isoprenoid part.[2] In order to investigate the structure-activity relationships of this compound, 5-geranyl and 5-tetradecyl derivatives (**2**) and (**3**) were prepared by the method as described previously (Scheme 2).[3] We were also interested in whether the absolute configuration of the pyran ring affects to the activity. Therefore, we stereoselectively synthesized a pair of enantiomers (2*R*,5*R*)- and (2*S*,5*S*)-**2** and a racemic *trans*-**3**.

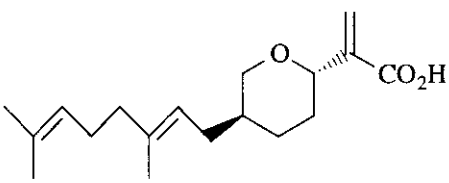
Scheme 1



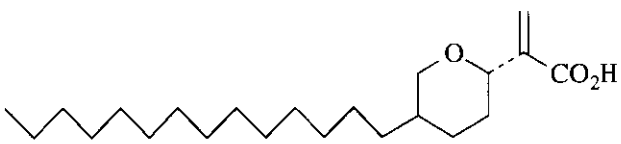
Rhopaloic acid A(2*R*,5*S*)-**1**



(2*R*,5*R*)-**2**



(2*S*,5*S*)-**2**

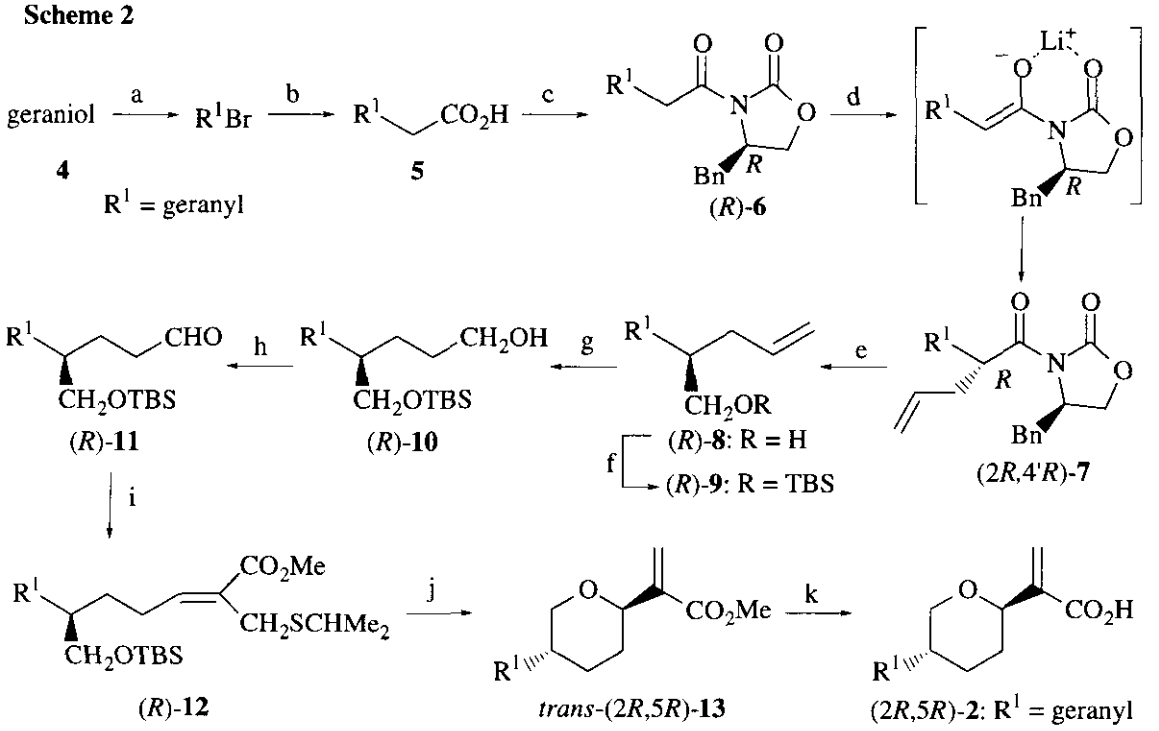


racemic *trans*-**3**

Carboxylic acid (**5**) was prepared by way of the malonic ester synthesis starting from geraniol (**4**). The Evans' asymmetric alkylation of *N*-acyl-oxazolidinone (**6**) was utilized for asymmetric construction of the stereogenic center in (2*R*,5*R*)-**2** (Scheme 2). The lithium enolate of (*R*)-**6** was treated with allyl bromide at −78 to 25 °C to give (2*R*,4'*R*)-**7**; $[\alpha]_D^{25}$ −47.0°; as a pure diastereomer (51% yield, 98% de).[4] Reduction of (2*R*,4'*R*)-**7** with $LiBH_4$ gave (*R*)-**8**; $[\alpha]_D^{25}$ +1.4°, quantitatively. The optical purity of the

allylation product [(*R*)-**8**] was established by conversion of the alcohol into its benzoate. The benzoate was analyzed on a DAICEL-OJ CHIRAL column (hexane). According to the chelation model of the enolate intermediate, the absolute configuration of (2*R*,4'*R*)-**7** was predicted to be 2*R*.[5] Following the protection of the alcohol [(*R*)-**8**] with a *tert*-butyldimethylsilyl group, the silyl ether [(*R*)-**9**] was subjected to regioselective hydroboration with 9-BBN reagent followed by oxidation with H_2O_2 to give (*R*)-**10** (84%). Swern oxidation of (*R*)-**10** was converted to (*R*)-**11** in 60% yield.

Scheme 2



Reagents and conditions: a. PPh_3 (1.2 eq), CBr_4 (1.2 eq), CH_2Cl_2, 0 °C, 30 min; b. 1) NaH (1.0 eq), $CH_2(CO_2Me)_2$ (4.0 eq), THF, 0 to 25 °C, 13 h, 80% (2 steps); 2) NaCl (1.0 eq), H_2O (2 eq), DMF, reflux, 20 h, 85%; 3) aq. 3 M KOH (excess), THF, reflux, 20 h, 91%; c. Et_3N (1.2 eq), PivCl (1.2 eq), CH_2Cl_2, then *N*-lithio-oxazolidin-2-one, THF, −78 to 25 °C, 10 h, 86%; d. LDA (1.1 eq), THF, −78 °C, 30 min, then allyl bromide (4 eq), −78 to 25 °C, 18 h, 51%; e. $LiBH_4$ (4 eq), THF, 0 °C, 3 h, 76%; f. TBSCl (1.2 eq), imidazole (2 eq), DMF, 3 h, 85%; g. 9-BBN (1.1 eq), THF, 0 to 25 °C, overnight, then aq. 7 M NaOH (4 eq), aq. 30% H_2O_2 (4 eq), 0 to 25 °C, overnight, 84%; h. DMSO (2 eq), $(COCl)_2$ (1.2 eq), Et_3N (5 eq), CH_2Cl_2, −60 °C, 12 h, 60%; i. NaH (1.1 eq), Me_2CHSH (1.1 eq), $(EtO)_2P(O)C(=CH_2)CO_2Me$ (1.2 eq), THF, 0 °C, 10 min, then (*R*)-**11**, 0 °C, 13 h, 59% (*E*/*Z* = 6/94); j. MeI (excess), $AgBF_4$ (3 eq), CH_2Cl_2, 25 °C, 1 h, then TBAF (3 eq), THF, 25 °C, 30 min, 28%; k. aq. 1 M LiOH (excess), THF, reflux, 18 h, 53%.

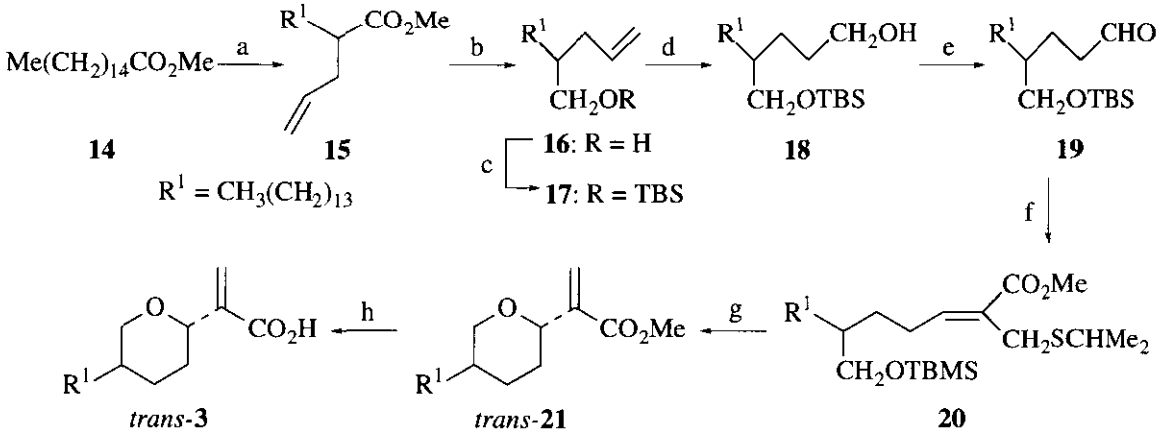
The modified Wittig-Horner-Emmons reaction of (*R*)-**11** with $(EtO)_2P(O)C(=CH_2)CO_2Me$ in the presence of $NaSCHMe_2$ at 0 °C furnished α,β-unsaturated ester [(*R*)-**12**], the *E*/*Z* ratio being 6/94. The geometry of the product was determined based on relative chemical shifts of the ^1H NMR signals and with DIF-NOE. When *3-H* of the *E*-isomer was irradiated, the intensity of 2-CH_2-S signal was enhanced with 8.2%. Reaction of *Z*-(*R*)-**12** with MeI/$AgBF_4$ followed by desilylation with TBAF afforded a mixture of *cis*- and

trans-tetrahydropyranylacrylate derivatives [(2*R*,5*R*)-**13**] in 28% yield (*cis*/*trans* = 1/9) in one pot reaction. The assignment of relative stereochemistry of *trans*-(2*R*,5*R*)-**13** was done by the ^{1}H NMR considerations: $J_{5,6}$-*ax* = 11.2 Hz, $J_{5,6}$-*eq* = 3.9 Hz. When *6-ax-H* at δ 3.17 was irradiated, the intensity enhancement of *2-H* at δ 4.02 by 8.2% was observed. The stereochemistry in the formation of pyranyl acrylate was rationalized by invoking a model of a chair-like transition state in which the long-chain alkyl group is located at the equatorial position. Hydrolysis of (2*R*,5*R*)-**13** in aqueous 1 M LiOH gave (2*R*,5*R*)-**2** in 53% yield.

The synthesis of the enantiomer [(2*S*,5*S*)-**2**] was accomplished by the same route as preparation of (2*R*,5*R*)-**2**. Allylation of (*S*)-**6** attached (*S*)-4-benzyloxazolidin-2-one as the chiral auxiliary by the same synthetic route to give (2*S*,4'*S*)-**7** (64%) in which the configuration at C-2 position was expected to be *S*. Successive conversion of (2*S*,4'*S*)-**7** afforded (2*S*,5*S*)-**2**; $[\alpha]_D^{25}$ −39.3°.

On the other hand, 5-tetradecyltetrahydropyranyl derivative (*trans*-**3**) was synthesized from methyl palmitate (**14**) by way of the analogous methodology as shown in Scheme 3. The geometry of *trans*-**3** was also determined on the basis of the NOE in ^{1}H NMR.

Scheme 3



Reagents and conditions: a. LDA (1.1 eq), THF, −78 °C, 30 min, then allyl bromide (4 eq), −78 to 25 °C, 18 h, 93%; b. $LiAlH_4$ (1.5 eq), THF, 0 °C, 3 h; c. TBSCl (1.2 eq), imidazole (2 eq), DMF, 3 h, 92% (2 step); d. 9-BBN (1.1 eq), THF, 0 to 25 °C, overnight, then aq. 12 M NaOH (4 eq), aq. 30% H_2O_2 (4 eq), 0 to 25 °C, overnight, 88%; e. DMSO (2 eq), $(COCl)_2$ (1.1 eq), Et_3N (5 eq), CH_2Cl_2, −60 °C, 12 h, 80%; f. NaH (1.4 eq), Me_2CHSH (1.3 eq), $(EtO)_2P(O)C(=CH_2)CO_2Me$ (1.2 eq), THF, 0 °C, 10 min, then **19**, 0 °C, 13 h, 61% (*E*/*Z* = 1/9); g. MeI (4 eq), $AgBF_4$ (2 eq), CH_2Cl_2, 25 °C, 5 h then TBAF (4 eq), THF, 25 °C, 14 h, 25%; h. aq. 1 M LiOH (excess), THF, reflux, 19 h, 73%.

The structure-activity relationships of rhopaloic acid A [(+)-**1**] as an inhibitor of gastrulation of sea urchin embryos were examined using the synthetic rhopaloic acid A, (2*R*,5*S*)-5-geranyl derivative (2*R*,5*R*)- and (2*S*,5*S*)-**2**, and 5-tetradecyl derivative (*trans*-**3**).[6] Natural type rhopaloic acid A (**1**), which was prepared by asymmetric synthesis,[3] inhibited the activity with the gastrulation of sea urchin embryos 50%-inhibition (IC_{50}) of about 0.52 μM, confirming it as the same degree of a potent inhibitor as the isolated sample (IC_{50}

0.5 μM) from a marine sponge, *Rhopaloeides* sp.[1] The related analogues (2*R*,5*R*)-**2** and (2*S*,5*S*)-**2** bearing a geranyl group more weakened the observed inhibition (IC_{50} 20 μM and 40 μM), respectively. The bioassay studies in racemic *trans*-**3** bearing a tetradecyl group showed drastically reduced activity (IC_{50} 320 μM).

ACKNOWLEDGMENTS

The measurements of NMR and HRMS were made using JEOL JNM-GSX270, JNM-LA500, and JMS-SX102A, respectively, at the Instrument Center for Chemical Analysis, Hiroshima University.

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4. All new compounds have been fully characterized by NMR and gave satisfactory exact MS. Spectral data for **2** and **3**: for **2**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.16-1.46 (m, 2H), 1.48-1.74 (m, 1H), 1.58 (s, 3H), 1.60 (s, 3H), 1.68 (s, 3H), 1.83-2.08 (m, 8H), 3.19 (t, *J* = 11.2 Hz, 1H), 4.06 (ddd, *J* = 11.2, 4.4, 2.0 Hz, 1H), 4.13 (d, *J* = 10.7 Hz, 1H), 5.05-5.14 (m, 2H), 5.93 (s, 1H), 6.38 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.1, 17.7, 25.7, 26.6, 29.7, 29.9, 31.7, 36.4, 39.8, 73.8(×2), 121.4, 124.2, 127.4, 137.2(×2), 143.4, 167.2; HRMS *m/z*: found 292.2027 [M^+] (Calcd for $C_{18}H_{28}O_3$ 292.2038); for **3**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.04-1.44 (m, 28H), 1.50-1.67 (m, 1H), 1.94 (d, *J* = 11.0 Hz, 2H), 3.16 (t, *J* = 11.3 Hz, 1H), 4.05 (ddd, *J* = 11.3, 4.0, 1.2 Hz, 1H), 4.13 (d, *J* = 11.3 Hz, 1H), 5.92 (s, 1H), 6.37 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 22.1, 26.6, 29.4, 29.5, 29.6(×4), 29.7(×3), 29.8, 30.3, 31.9, 32.3, 35.5, 74.1, 76.2, 126.8, 140.8, 169.3; HRMS *m/z*: found 352.3019 [M^+] (Calcd for $C_{22}H_{40}O_3$ 352.2977).
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