COMMUNICATIONS TO THE EDITOR

Convenient Total Synthesis of NM-3, an Antiangiogenesis Agent, and Its Optical Resolution

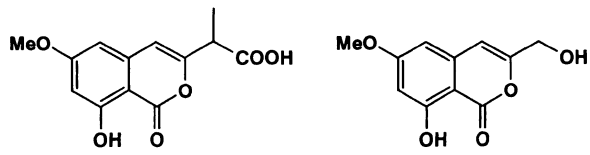
Sir:

During our studies on the screening program for new antitumor drugs, we found a novel microbial product, cytogenin (**2**), isolated from a culture of *Streptoverticillum eurocidium*. Cytogenin exhibited moderate antitumor activity both *in vivo* and *in vitro*[1] and has been also demonstrated to have efficacy against animal models for human rheumatoid arthritis, including type II collagen-induced arthritis in mice and adjuvant arthritis in rats.[2] As mechanism of angiogenesis is closely related to both tumor growth and rheumatoid arthritis, we intended to examine the antiangiogenic effects of cytogenin using mouse dorsal air sac assay system.[3] We have also synthesized cytogenin derivatives and related compounds to enhance the stability *in vivo*, and found 2-(8-hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran-3-yl)propionic acid (NM-3; **1**) to be the most stable and has excellent antiangiogenic activity.[4] In view of the biological activity and synthetic utility as intermediates, several methods have been reported for preparing the isocumarin compounds.[5] From the retrosynthetic perspective, NM-3 is expected to be synthesized from two components (**3** and **5** or **4** and **6**) as depicted in Fig. 2. In this communication we describe the two efficient methods of cyclization applicable to industrial synthesis of NM-3 and resolution of each enantiomer.

Preparations of isocoumarin compounds from homophthalic acid and acetic anhydride have been described by HILL *et al.*[6] The feature of this reaction is the acylation and decarboxylation of the first intermediate anhydride of homophthalic acid. We first explored the isocoumarin preparation from homopthatic acid and malonic acid derivatives. (Scheme 1) Orsellinic acid dimethylether was efficiently transformed to 3,5-dimethoxy homophthalic acid (**3**) by treatment with lithium diisopropylamide and dimethyl carbonate according to HAUSER's procedures.[7] Protected NM-3 intermediate (**7**) was prepared in excellent yield by treatment of **3** with ethyl methylmalonyl chloride (**5**) in the presence of triethylamine.

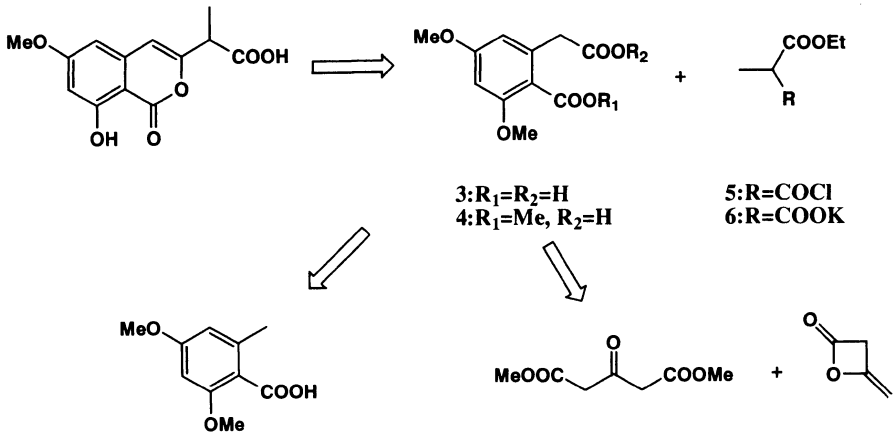
The next exploration was accomplished by the elongation and subsequent cyclization. (Scheme 2) The homophthalic half ester (**4**) was obtained by the condensation of dimethyl

Fig. 1. Structures of NM-3 (**1**) and cytogenin (**2**).

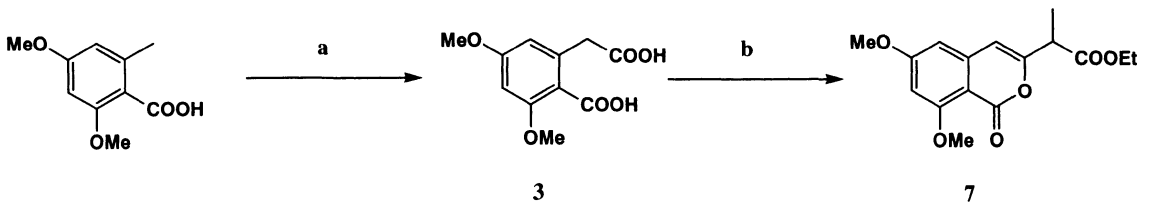


1:NM-3 **2:cytogenin**

Fig. 2. Retrosynthetic analysis of NM-3.



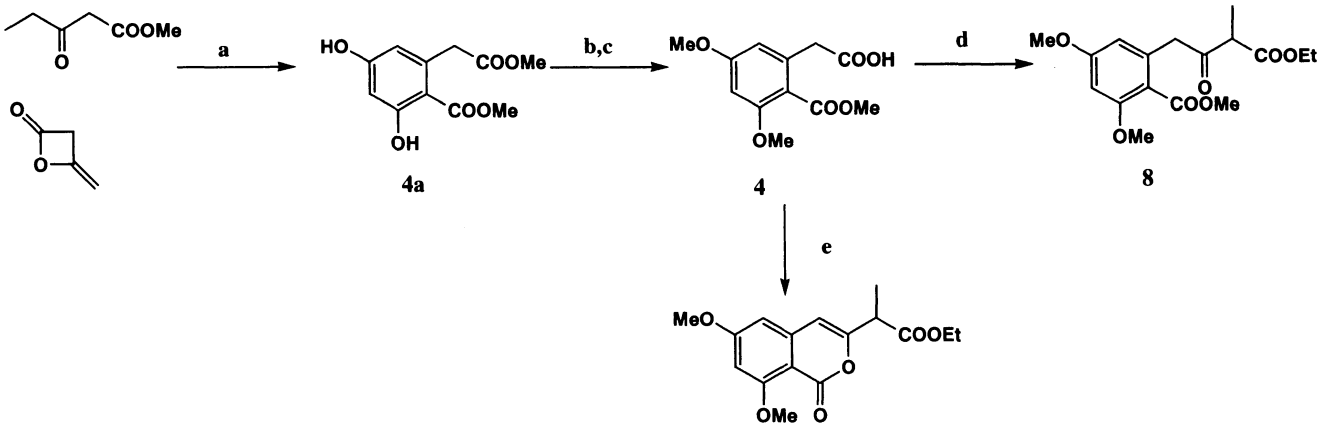
Scheme 1.



3 7

Conditions; (a) LDA/THF, -70°C; then CO(OMe)2, -30°C, 92%. (b) 5(3.0eq.), Et3N(3.75eq.)/CH_2Cl_2, 0°C, 89%.

Scheme 2.



4a 4 8 7

Conditions; (a) Ref 9, (b) MeI(4eq.), K_2CO_3(3eq.)/DMF, r.t., no purification, (c) 1 mol/l(1.2eq.)/MeOH, r.t., 83% from 4a (d) CDI(1.1eq.)/THF, r.t; then 6(2.1eq.), Et_3N(2.0eq.), $MgCl_2$(2.5eq.)/THF, r.t., 63% (7:3%), (e) CDI(1.1eq.)/CH_3CN, r.t; then 6(2.1eq.), Et_3N(2.0eq.), $MgCl_2$(2.5eq.)/CH_3CN, r.t.;then reflux, 83%.

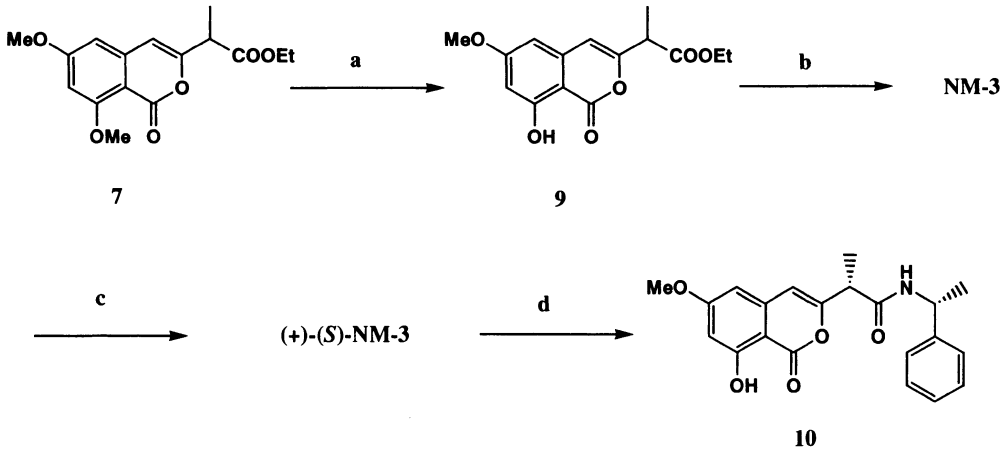
acetonedicarboxylate and diketene,[8] followed by protection of the hydroxyl groups as methyl ethers and selective hydrolysis of the side chain ester. The elongation process was accomplished by MASAMUNE's procedures,[9] as follows. After activation of **4** by *N,N'*-carbonydiimidazole, the resulting imidazolide was treated with ethyl methylmalonate potassium salt (**6**) in the presence of magnesium chloride and triethylamine at room temperature afforded elongated compound (**8**) and cyclic compound (**7**) in 65% and 3%, respectively. Based on this result, the one-pot reaction of elongation and cyclization was expected. After the elongation reaction, the cyclization proceeded smoothly by refluxing the reaction mixture to generate desired compound (**7**) in 70% yield. The reaction yield was improved to 83% by changing the reaction solvent THF to MeCN.

Selected demethylation of methyl aryl ether proceeded smoothly with boron tribromide in methylene chloride. This

7: ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.46 (1H, d, J=2.2 Hz), 6.38 (1H, d, J=2.2 Hz), 6.25 (1H, s), 4.19 (2H, q, J=7.0 Hz), 3.96 (3H, s), 3.89 (3H, s), 3.57 (1H, q, J=7.3 Hz), 1.53 (3H, d, J=7.3 Hz), 1.26 (3H, t, J=7.0 Hz). FAB-MS (*m/z*): 307 $(M+H)^+$

9: ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.03 (1H, σ), 6.46 (1H, d, J=2.2 Hz), 6.38 (1H, d, J=2.2 Hz), 6.34 (1H, s), 4.20 (2H, q, J=7.0 Hz), 3.87 (3H, s), 3.59 (1H, q, J=7.3 Hz), 1.54 (3H, d, J=7.3 Hz), 1.27 (3H, t, J=7.0 Hz). FAB-MS (*m/z*): 293 $(M+H)^+$

Scheme 3.



Conditions; (a) $MgCl_2$(2.0eq.), KI(2.0eq.)/THF, reflux, 97%, (b) 1mol/l NaOH(3.0eq.)/CH_3CN-MeOH(1:1), 0℃, quant. (c) 1.(*R*)-phenlethylamine resolution by fractional crystallization 2. 1mol/l HCl-acetone-MeOH (d) (*R*)-phenylethylamine/WSC.

Fig. 3. ORTEP drawing of **10**.

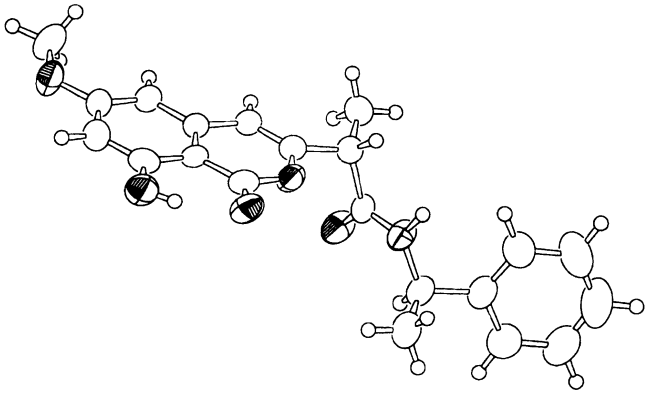


Table 1. Crystal data of **10**.

Empirical formula	**$C_{21}H_{21}NO_5$**
Formula weight	**367.40**
Crystal system	**triclinic**
Space group	**P1(≠1)**
Lattice Parameters:	**a = 12.661(1) Å**
	b = 15.486(2) Å
	c = 4.869(1) Å
	α = 95.04(1)°
	β = 96.82(1) °
	γ = 94.837(9) °
	V = 939.9(2) Å^3
Z	**2**
Dcalc.	**1.298 g/cm^3**
μ(CuKα)	**7.67 cm^{-1}**

procedure, however, needed careful control of the reaction conditions to avoid demethylated at the 6 position to produce the 6,8-dihydroxyisocumarin. The cleavage of methyl aryl ether containing intermolecular hydrogen bonds was carried out with expensive reagents such as magnesium iodide.[10] During the examination of the demethylation reaction, we found an effective and selective monodemethylation procedure as follows. Refluxing the THF solution of (**7**) with magnesium chloride and potassium iodide gave the corresponding demethylated product (**9**) in 97% yield without producing the 6,8-dihydroxycompound. Because of the neutral reaction condition and the low cost of the reagent, the use of magnesium chloride and potassium iodide will be particularly attractive for the cleavage of activated methyl aryl ethers. (Scheme 3) Finally, the hydrolysis of **9** with 3 equiv. of NaOH at 0°C furnished **1** in quantitative yield.

Racemic NM-3 was resolved by twice fractional

crystallization of the diastereomeric salts formed by addition of 1 equiv. of (*R*)-1-phenylethylamine to a solution of NM-3 in THF. The optically pure (+)-NM-3[*1] [99.4% ee (HPLC analysis), $[\alpha]_D^{18}$ +29.0° (*c* 0.5, MeOH)] was obtained by precipitation from a 1 mol/liter HCl - acetone - MeOH solution of the single diastereomeric salt. The absolute configuration was determined by X-ray analysis[*2] of (+)-NM-3 amide derivative (**10**) obtained by treatment with (*R*)-phenylethylamine and water soluble carbodiimide (WSC: 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide). (Scheme 3) The amide derivative (**10**) was recrystallizated from MeOH solution to give colorless prism crystals. As a result, the absolute configuration of (+)-NM-3 was confirmed to be the *S*-form. The ORTEP drawing of **10** is shown in Fig. 3. The crystal data are summarized in Table 1.

The antiangiogenic effects of racemic NM-3 and either of two enantiomers in mouse dorsal air sac assay system indicated that the enantiomers were essentially equipotent.

In conclusion, we have achieved a convenient total synthesis of NM-3 from homophalic derivatives with a useful demethylation of activated methyl aryl ethers. The absolute configuration of (+)-NM-3 was determined to be *S* by X-ray crystallographic analysis.

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*1 (−)-(*R*)-NM-3: 99.4% ee (HPLC analysis), $[\alpha]_D^{18}$ −27.6° (*c* 0.5, MeOH) HPLC condition: (Column: CHRALPAK OJ-R (4.6×150 mm) (DAICEL CHEMCAL INDUSTRIES, LTD), mobile phase: MeOH - H_2O - AcOH (70 : 30 : 1), flow rate: 0.5 ml/minute, detect: 244 nm).

*2 A colorless prism crystal of $C_{21}H_{21}NO_5$ having approximate dimensions of 0.08×0.15×0.40 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation and a rotating anode generator. Of the 3199 reflections, which were collected, 2811 were unique. No decay correction was applied. The structure was solved by direct methods[11] and expanded using Fourier techniques[12]. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full matrix last-squares refinement was based on 2695 observed reflections ($I>1.00\sigma$ (I)) and 484 variable parameters and converged with unweighted and weighted agreement factors of $R=0.041$ and $Rw=0.053$. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.15 and $-0.23e^-/Å^3$, respectively. The ORTEP drawing is shown in Fig. 3. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation.