SYNTHESIS OF A NEW POTENT ANTI-ANGIOGENIC AGENT, 17α-ACETOXY-9α-FLUORO-6α-METHYLPROGESTERONE (9α-FLUOROMEDROXYPROGESTERONE ACETATE [FMPA])

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A new anti-angiogenic agent, 17α-acetoxy-9α-fluoro-6α-methylprogesterone (9α-fluoromedoroxyprogesterone acetate [FMPA, **9**]) was synthesized in a 10-step sequence. FMPA (**9**) had about two orders of magnitude stronger anti-angiogenic activity than medroxyprogesterone acetate (MPA), as estimated in a bioassay involving chorioallantoic membranes of growing chick embryos.

KEY WORDS 9α-fluoromedroxyprogesterone acetate; synthesis; anti-angiogenic activity; anti-cancer agent; angiogenic diseases

It has been recognized that angiogenesis, or the new blood vessel development, is an attractive target for the treatment of various diseases, including cancer, AIDS, diabetic retinopathy and rheumatoid arthritis, since neovascularization plays a critical role in the induction and/or maintenance of these so-called angiogenic diseases.[1)] From this point of view, the development of useful drugs suppressing angiogenesis is a major focus in the treatment of such diseases.

17α-Acetoxy-6α-methylprogesterone (medroxyprogesterone acetate [MPA]) is widely used in endocrine therapy for breast cancer and other diseases without unacceptable side effects.[1c)] We previously suggested the involvement of the anti-angiogenic effect of MPA in its antitumor action in an *in vivo* system involving autochthonous rat mammary tumors induced by 7,12-dimethylbenz[*a*]-anthracene (DMBA),[2a)] a useful experimental model system for studying therapies for breast cancer.[1c)] This could explain why MPA exhibits antitumor activity *in vivo* but not *in vitro*, indicating that its effect is host mediated.[1c)] We also showed that it affects angiogenesis in a bioassay system involving growing chick embryo chorioallantoic membrane (CAM).[2b)] This CAM assay also revealed that 9α-fluoro-16α-methylpredonisolone (dexamethasone) had the potent anti-angiogenic activity.[2c)] It is well known that fluorination often results in potentiation of biological activity and/or bioavailability of various compounds.[3)] Based on these findings, we proposed that the anti-angiogenic effect might be increased by the design of novel MPA having a fluorine atom at the C-9α position. To substantiate this hypothesis, we attempted to synthesize 9α-fluorinated MPA (FMPA, **9**) and examined its anti-angiogenic potency in a bioassay system.

For the synthesis of 9α-fluoro-MPA, we planned to utilize the 11β-hydroxy group of the steroid nucleus according to the method of Dadson[4)] and then chose 4-pregnen-11β, 17α-diol-3, 20-dione (**1**)

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as the starting material. After the 11β-hydroxy group of **1** was protected by acetylation, ketalization of **2** followed by Grignard reaction with MeMgBr gave the 5α-hydroxy-6β-methylpregnene **4**. Subsequent treatment of **4** with $KHSO_4$ in an aqueous acetone followed by treatment with NaOH in an aq. methanol in order to avoid dehydration of the 11β-OH group afforded the 6β-methylpregnene **6** (23% from **1**). The fluorination of the key step to the 9α-position of **6** was carried out by 70% HF in pyridine [4] to give the 9α-fluoroprogesterone **7** (23%) together with dehydrated product. 6β-Methylprogesterone **7** was converted into the 6α-methylprogesterone **8** in the usual manner [5] (58%). Finally, acetylation of **8** with the mixed anhydride method [6] produced 9α-fluorinated MPA (**9**) (36%). Thus the total synthesis of 17α-acetoxy-9α-fluoro-6α-methylprogesterone (9α-fluoromedoroxyprogesterone acetate [FMPA, **9**]) was completed in a 10-step sequence as depicted in Chart 1. The structure of FMPA (**9**) was fully characterized by spectroscopic evidence [7] and X-ray analysis. Although the overall yield in 10 steps was low (1.1%), improved synthesis of FMPA (**9**) using a cheaper starting material than **1** is now in progress.

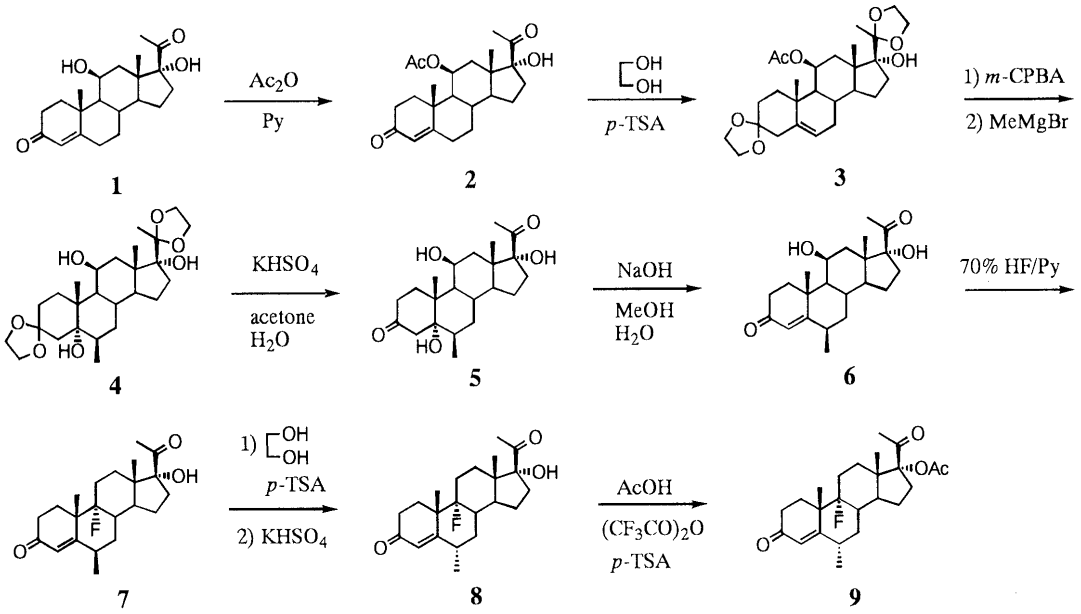

Chart 1

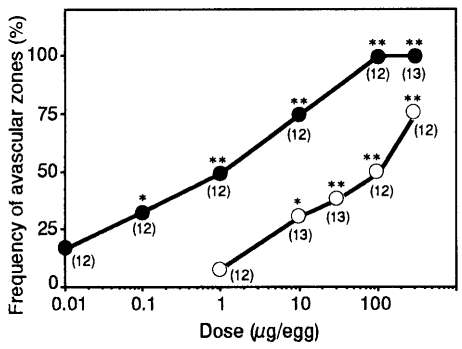

Fig. 1. Inhibitory Effects of MPA and FMPA on Embryonic Angiogenesis

4.5-Day-old CAMs were treated with increasing doses of MPA (○) or FMPA (●) for 2 days. Anti-angiogenic activity was determined by measuring the avascular zone. The values in parentheses are the numbers of CAMs examined. * $P<0.01$ compared to the control CAMs (n=26), which did not show an avascular zone; ** $P<0.001$ compared to the control.

The anti-angiogenic activity was determined in a CAM assay system as described previously.[8a,b] Briefly, 4.5-day-old CAMs were treated with various doses of FMPA (**9**) or MPA in a humidified egg incubator at 37°C for 2 days, after which an appropriate volume of 20% fat emulsion was injected into the chorioallantois to depict the vascular network more clearly. When the test sample caused an avascular zone of longer than 3 mm in diameter in the CAM examined, the anti-angiogenic response was assessed as being effective. Results of anti-angiogenic activity were analyzed by Fisher's exact probability test,

with $P<0.05$ as the level of significance.

The dose-response relationships for the appearance of an avascular zone are shown in Fig. 1. Treatment with MPA caused a dose-dependent angiogenesis-inhibitory activity with a minimum effective dose required for causing an avascular zone of 10 μg/egg and an ID50 value of 100 μg/egg, which reconfirmed our previous observation.[2b] FMPA (**9**) treatment resulted in more potent inhibition of embryonic angiogenesis. Its powerful anti-angiogenic effect was dose dependent, and the minimum effective dose and the ID50 value were 0.1 and 1 μg/egg, respectively. At doses of over 100 μg/egg FMPA (**9**) produced significant avascular zones in all of the treated CAMs.

FMPA (**9**) has 100 times greater anti-angiogenic activity than MPA in this bioassay system. This fact might imply that FMPA (**9**) exhibits a more powerful inhibitory activity against the angiogenic response triggered by DMBA-induced rat mammary carcinomas, thereby exerting a stronger antitumor effect on these mammary carcinomas than the parent compound.

Although the mechanism of the anti-angiogenic action of MPA has not yet been fully established, the effects of FMPA (**9**) on functions of vascular endothelial cells related to angiogenesis are under investigation in comparison with those of MPA.[8c]

In conclusion, we synthesized FMPA (**9**) as a promising candidate for an angiogenesis-targeting drug on the basis of our hypothesis outlined above. Further investigations on the mechanism of the anti-angiogenic action of FMPA (**9**) and its efficacy against different types of angiogenesis-dependent diseases like cancer seem to be worthwhile.

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7) FMPA (**9**): mp 208-210°C (EtOAc/hexane), $[\alpha]_D^{20}$+36.0°(c=0.210, $CHCl_3$); ^{1}H-NMR (500MHz, $CDCl_3$) δ: 0.68 (3H, s), 1.12 (3H, d, J=6.5Hz), 1.31 (3H, s), 2.05 (3H, s), 2.10 (3H, s), 2.96 (1H, ddd, J=2.5, 11.3, 14.3Hz), 5.90 (1H, d, J=1.5Hz); ^{13}C-NMR (125MHz, $CDCl_3$) δ : 13.34 (C-18), 1.95 (6α–Me), 21.08 ($\underline{C}H_3COO$), 21.28 (C-19), 23.69 (C-15), 24.95 (C-11), 26.18 (C-21), 26.76 (C-12), 29.1 (C-1), 30.28 (C-16), 33.07 (C-6), 33.55 (C-2), 34.39 (C-7), 37.59 (C-8), 43.26 (C-10), 44.58 (C-14), 46.0 (C-13), 96.24 (C-17), **99.42 (C-9)**, 123.92 (C-4), 169.92 (C-5), 170.60 ($CH_3\underline{C}OO$), 196.68 (C-3), 203.63 (C-20); HRMS m/z: 404.2377 (Calcd for $C_{24}H_{33}O_4F$: 404.2363).

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