Preparation and Antirheumatic Activity of Optically Active 2-Acetylthiomethyl-4-(4-methylphenyl)-4-oxobutanoic Acid (KE-298)

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2-Acetylthiomethyl-4-(4-methylphenyl)-4-oxobutanonic acid (KE-298) is an antirheumatic agent. To elucidate the effects of optically active KE-298, we resolved the racemic acid and obtained the two optical isomers. (+)-KE-298 was converted to the 4-bromobenzyl ester derivative and the absolute structure was confirmed as (*S*) by X-ray crystallographic analysis.

The pharmacological activities of the optical isomers and racemic KE-298 were compared by using the characteristic tests for KE-298. Though (+)-KE-298 showed a stronger suppressive effect on rat adjuvant arthritis than (−)-KE-298, no difference between the two isomers was detected in *in vitro* tests (enhancing effect on lymphocyte transformation, IL-1 antagonistic effect).

Key words 2-acetylthiomethyl-4-(4-methylphenyl)-4-oxobutanonic acid; antirheumatic activity; optical resolution; absolute stereochemistry

In a previous paper,[1] we reported that 4-phenyl-4-oxobutanoic acid derivatives with a mercapto moiety have potent antirheumatic effects. In further pharmacological studies, 2-acetylthiomethyl-4-(4-methylphenyl)-4-oxobutanoic acid (KE-298, Chart 1) was found to have properties which make it suitable for clinical use.

In this study, we prepared optically active KE-298 by optical resolution and examined the biological activities of the enantiomers.

Preparation of Optically Active KE-298 Using 0.6 equivalent of cinchonidine with (±)-KE-298 in ethyl acetate, the cinchonidine salt of (+)-KE-298 was separated as colorless crystals, and the salt was recrystallized from ethyl acetate. (−)-KE-298 was obtained as the (*R*)-(+)-1-phenylethylamine salt from the mother liquid, and the salt was recrystallized from dichloromethane-*n*-hexane. From the salt, (+)- or (−)-KE-298 was extracted by partitioning between ethyl acetate and aqueous 10% hydrochloric acid. The specific rotations of (+)- and (−)-KE-298 were +28.3° and −28.7°, respectively ($[\alpha]_D^{25}$, $c=1.5$, chloroform).

To determine the absolute structure, (+)-KE-298 was converted to the 4-bromobenzyl ester derivative by reacting it with 4-bromobenzyl bromide in the presence of potassium bicarbonate in *N,N*-dimethylformamide (DMF) at room temperature (89%, $[\alpha]_D^{25}$ −5.2° ($c=1.0$, chloroform)). The absolute stereochemistry of the compound was confirmed by X-ray crystallographic analysis to be (*S*).

Biological Activities Characteristic pharmacological effects of KE-298 include (1) a suppressive effect on rat adjuvant arthritis,[1a] (2) an enhancing effect in the lymphocyte transformation test,[1b] and (3) an IL-1 antagonistic effect.[2]

We compared the pharmacological activities among optical isomers, (±), *S*-(+), *R*-(−), with the following results.

(1) The racemic ((±)-), *S*-(+)- and *R*-(−)-isomer showed a suppressive effect on rat adjuvant arthritis. The order of potency was (+)>(±)>(−), as shown in Fig. 1.

(2) It has been reported that LPS-induced lymphocyte transformation in adjuvant arthritis rats is depressed, as it is in rheumatoid arthritis patients.[3] As KE-298 enhanced the LPS-induced lymphocyte transformation by the induction of IL-2 receptor expression, it was suggested that the enhancing effect of KE-298 on depressed lymphocyte function contributes to the suppressive effect of KE-298 on rat adjuvant arthritis. In these experiments, as shown in Fig. 2, the degree of enhancing effect by the two optical isomers was the same.

(3) In rheumatoid arthritis patients, IL-1 production is induced in synovial cells and peripheral blood, and IL-1 elicits various inflammatory symptoms, such as the proliferation of synovial cells, the stimulation of osteoclasts, and the degradation of cartilage and bone.[4] KE-298 is an IL-1 antagonist, as shown in Fig. 3, but no difference in activity between the optical isomers was detected.

We could not find any difference between the optical isomers as regards *in vitro* activities, which are considered to be related to antirheumatic activities. The stereoselectivity in the pharmacokinetics of KE-298 was studied by Yoshida *et al.*[5] The blood levels of radioactivity after oral administration of [^{14}C]*S*-(+)-KE-298 in rats were found to be approximately twice as high as those after [^{14}C]*R*-(−)-KE-298. Their pharmacokinetic differences seem to be one of the reasons for the difference in the degree of the suppressive effect on rat adjuvant arthritis.

Experimental

All melting points (mp) are uncorrected. Infrared (IR) spectra were measured using a JASCO DS-301 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were taken in $CDCl_3$ at 200 MHz with tetramethylsilane (TMS) as an internal standard, on a Varian XL-200 spectrometer. The chemical shifts are expressed as ppm downfield from

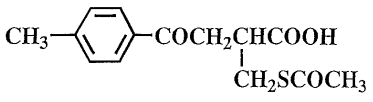

Chart 1. 2-acetylthiomethyl-4-(4-methylphenyl)-4-oxobutanoic acid

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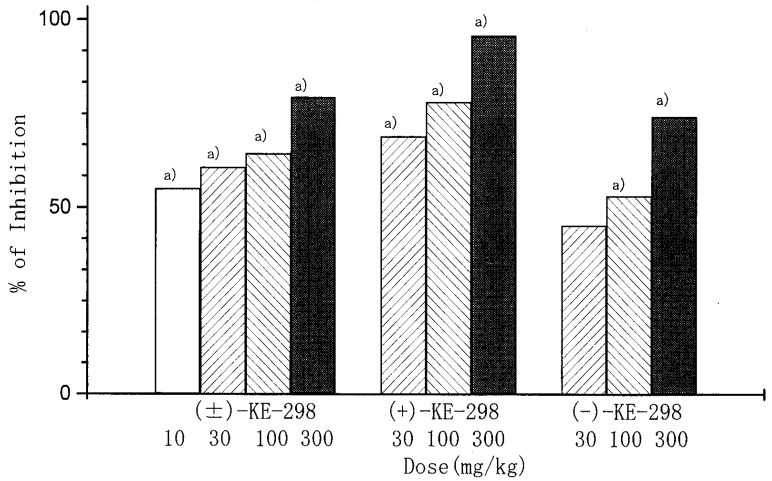

Fig. 1. Effect of Optical Isomers of KE-298 on Edema Volume of Foot Pads of Rats with Developing Adjuvant Arthritis (AA)

Significantly different from the control, a) $p<0.01$.

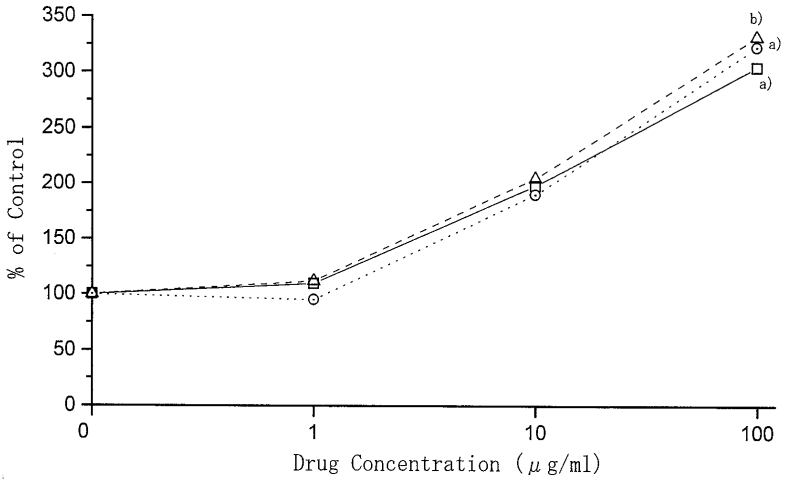

Fig. 2. Effect of Drugs on LPS-Induced Lymphocyte Transformation

Significantly different from the control, a) $p<0.05$; b) $p<0.01$. —□—, (±)-KE-298; ···⊙···, (+)-KE-298; —△—, (−)-KE-298.

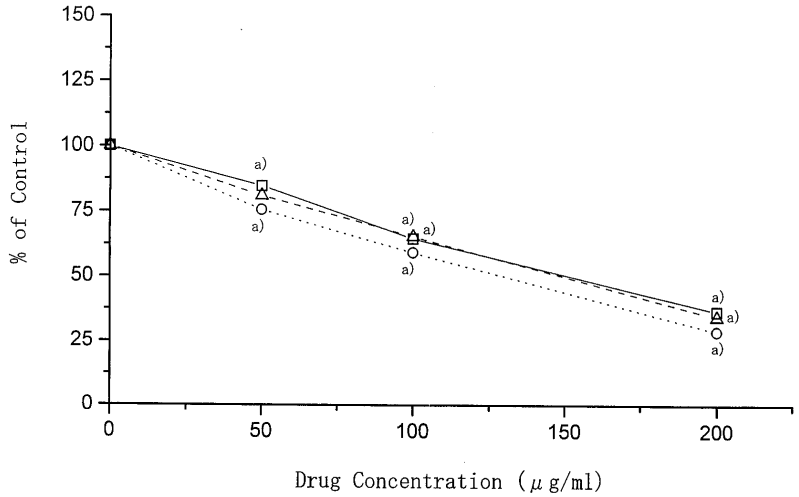

Fig. 3. Effect of Drugs on IL-1-Induced Proliferation of Thymocytes

Drugs were added at the same time as IL-1. Data are % of control. Significantly different from the control, a) $p<0.01$. —□—, (±)-KE-298; ···○···, (+)-KE-298; —△—, (−)-KE-298.

TMS. The following abbreviations are used: s = singlet and m = multiplet. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. HPLC was performed on an HPLC Chiralcel OD (Daicel Chemical Co.) (flow, 10 ml/min, UV, 270 nm; temperature, 40 °C). *n*-Hexane–2-propanol–formic acid (180:20:1, v/v) was used as the eluent.

Optical Resolution of KE-298 (±)-2-Acetylthiomethyl-4-(4-methylphenyl)-4-oxobutanoic acid ((±)-KE-298) (50.0 g, 178 mmol) and cinchonidine (31.5 g, 107 mmol) were dissolved in MeOH (200 ml) and the solution was concentrated under reduced pressure. The residue was crystallized from AcOEt (500 ml) to give colorless needles, which were

added to fresh AcOEt (500 ml). The mixture was heated under reflux with stirring for 5 min, then allowed to cool to obtain purified needles (39.9 g) ($[\alpha]_D^{25}$ −45.4° (c=1.5, chloroform)). The needles were partitioned between AcOEt (500 ml) and 10% aqueous HCl (100 ml). Then the organic layer was washed with H_2O and brine, dried ($MgSO_4$), and concentrated. The residue was recrystallized from Et_2O (150 ml)–n-hexane (250 ml) to yield (+)-KE-298 (15.8 g, 31.5% from (±)-KE-298)), which showed 100% optical purity on HPLC analysis, mp 87—89 °C, $[\alpha]_D^{25}$ +28.3° (c=1.5, chloroform).

The mother liquid (AcOEt) was washed with 10% aqueous HCl (100 ml), H_2O and brine, then dried ($MgSO_4$) and concentrated. The residue was dissolved in AcOEt (400 ml) and mixed with a solution of (*R*)-(+)-1-phenylethylamine (10.9 g, 89.9 mmol) in AcOEt (50 ml). The precipitate was dissolved in CH_2Cl_2 (170 ml) and crystallized by adding n-hexane (600 ml), at room temperature, to give colorless needles (26.3 g). ($[\alpha]_D^{25}$ −1.1° (c=1.5, chloroform)). The needles were treated with a mixture of AcOEt (500 ml) and 10% aqueous HCl (100 ml). The organic layer was washed with H_2O and brine, then dried ($MgSO_4$) and concentrated. The residue was recrystallized from Et_2O (150 ml)–n-hexane (250 ml) to yield (−)-KE-298 (14.9 g, 29.8% from (±)-KE-298, mp 87—89 °C, $[\alpha]_D^{25}$ −28.7° (c=1.5, chloroform)), which showed 100% optical purity on HPLC analysis. mp 87—89 °C. Chiral HPLC: t_R, (+)-KE-298 (10.5 min), (−)-KE-298 (12.4 min).

Preparation of 4-Bromobenzoyl (*S*)-(−)-2-Acetylthiomethyl-4-(4-methylphenyl)-4-oxobutanoate A stirred solution of (*S*)-(+)-KE-298 (2.80 g) and 4-bromobenzyl bromide (2.80 g, 11.2 mmol) in DMF (30 ml) was treated with K_2CO_3 (1.40 g, 10.1 mmol), and the stirring was continued for 1 h at room temperature. The solution was diluted with H_2O, and was extracted with Et_2O. The organic layer was washed with 10% aqueous HCl, H_2O and brine, then dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography using Et_2O–n-hexane (1 : 5, v/v) as an eluent, and the product was crystallized from toluene–n-hexane to give colorless plates (4.00 g, 89%), $[\alpha]_D^{25}$ −5.2° (c=1.0, $CHCl_3$), mp 51—52 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.32 (3H, s), 2.42 (3H, s), 3.09—3.55 (5H, m), 5.08 (2H, s), 7.22 (4H, m), 7.45 (2H, d, J=8 Hz), 7.82 (2H, d, J=8 Hz). IR (KBr) cm^{-1}: 2914, 1730, 1697, 1676, 1606, 1167, 815. *Anal.* Calcd for $C_{21}H_{21}BrO_4S$: C, 56.13; H, 4.71. Found: C, 55.98; H, 4.73.

X-Ray Crystal Structure Analysis $C_{21}H_{21}BrO_4S$, M_r=449.37, monoclinic, space group $P2_1$, a=12.999 (2)Å, b=8.840 (2)Å, c=9.817 (1)Å, β=117.07 (1), V=1052.7 (3)Å^3, Z=2, D_c=1.42 gcm^{-3}, μ=35.58 cm^{-1}. R=4.1% over 1887 independent reflections.

The structure was determined by direct methods using SHELXS86.[7] The absolute configuration of (−)-4-bromobenzyl 2-acetylthiomethyl-4-(4-methylphenyl)-4-oxobutanonate was determined by the R-value method from the anomalous scattering due to the bromine atom and was found to be (*S*).[8] Full lists of fractional atomic co-ordinates, bond lengths and angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Center.

Lymphocyte Transformation Test (LTT)[9] Female BDF_1 mice were exsanguinated and the spleens were excised and placed in sterile Petri dishes containing cold RPMI 1640 medium (Gibco Laboratories, U.S.A.). Spleen cell suspensions were prepared by gently teasing the cells with needles followed by hemolysis. The cells were washed twice and suspended in culture medium (RPMI 1640 medium supplemented with 100 u/ml penicillin, 100 μg/ml streptomycin and 10% heat-inactivated fetal calf serum). The cells were cultured in microtiter plates (Nunc Co., U.S.A.), with each well containing 2×10^5 spleen cells in 0.05 ml of the culture medium, 0.2 μg LPS in 0.05 ml of the culture medium, and the drug in 0.1 ml of the culture medium or 0.1 ml of culture medium alone. The culture was carried out in 95% O_2/5% CO_2 at 37 °C for 48 h and pulsed with 0.25 μCi of methyl-^{3}H-thymidine (^{3}H-TdR, 5.0 Ci/mmol, Amersham Co., Japan) during the final 22 h. The cells were harvested on glass fiber filter paper, using a multiple cell harvester. Uptake of ^{3}H-TdR was determined by liquid scintillation spectroscopy.

IL-1-Induced Thymocyte Proliferation (Assay for IL-1 Antagonist Activity)[10] Thymocytes (1.5×10^6) from C3H/Hej mice suspended in 0.05 ml of culture medium were added to 96-well plates. Test compounds at varying concentrations, 6 u/ml IL-1 and 12 μg/ml PHA (final concentrations) were added. After 48 h of incubation, the cells were pulsed with ^{3}H-TdR and harvested at 16 h. Total ^{3}H-TdR was determined by liquid scintillation spectroscopy. Inhibition of ^{3}H-TdR uptake consequent upon IL-1-induced thymocyte proliferation, as compared to the control, revealed the antagonist activity of the test compounds.

Adjuvant-Induced Arthritis[11] SD rats were inoculated intracutaneously with 0.6 mg of heat-killed *Mycobacterium butyricum* (Difco Laboratories, U.S.A.) suspended in 0.1 ml of liquid paraffin and were examined for 18 d. The edema volume of the hind paws was measured using a plethysmograph (volume meter, MK-500, Muromachi Kikai Co., Japan).

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