Reactions of 2-Guanidino-1-cyclohexanol with Ketals and Acetals

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Reactions of 2-guanidino-1-cyclohexanols (**1**, $\mathbf{1}_d$, $\mathbf{1}_l$) with 1,1-dimethoxycyclohexane in *N,N*-dimethylformamide in the presence of *p*-toluenesulfonic acid gave the *N,O*-cyclohexylidene derivatives (**5**, $\mathbf{5}_d$, $\mathbf{5}_l$), respectively. Similar reactions of $\mathbf{1}_l$ with 2,2-dimethoxypropane and with dimethyl acetals of *p*-tolualdehyde and *p*-bromobenzaldehyde also gave an *N,O*-isopropylindene, *N,O*-(*p*-methylbenzylidene) and *N,O*-(*p*-bromobenzylidene) derivative ($\mathbf{8}_l$, $\mathbf{9}_l$, $\mathbf{10}_l$), respectively. From IR and PMR spectral studies of $\mathbf{9}_l$ *p*-toluenesulfonate, seven-membered structures as exemplified by **9A** were assigned. The *N,O*-blocked derivatives (**5**,**9**) are stable in weakly acidic or alkaline media, but in stronger media, they are cleaved to **1**. The usefulness of the protective groups was also examined. **9** was found to resist tosylation or mesylation in pyridine but not acylation. The numerical values of optical rotations of the guanidinocyclohexanols were greatly enhanced by *N,O*-blocking.

During the course of synthetic studies on dihydrostreptomycin and related compounds, we undertook to protect the hydroxyl groups of the antibiotic by conversion into acetals or ketals. When 2″-*N*-benzyloxycarbonyldihydrostreptomycin[1] was treated with 1,1-dimethoxycyclohexane, a ketal reagent, in *N,N*-dimethylformamide (DMF) in the presence of acid catalyst, we obtained a tetracyclohexylidenated derivative. The product was found to contain no *O*-(1-methoxycyclohexyl) group[6] as judged by the PMR spectrum, although it was not isolated in crystalline state. This fact was unusual, since the starting material has only three sets of hydroxyl groups for the ketal formation. The fourth cyclohexylidene group might be formed between the guanidino and hydroxyl groups in the streptidine moiety of the dihydrostreptomycin. In order to clarify the above structure, we chose *trans*-2-guanidino-1-cyclohexanol (**1**) as a model compound and treated it with 1,1-dimethoxycyclohexane in the presence of acid catalyst. Since the compound has *trans*-diequatorial guanidino and hydroxyl groups attached to a cyclohexane ring, the compound can be deemed as a model compound for the streptidine portion of dihydrostreptomycin.

The *dl-trans*-2-guanidino-1-cyclohexanol (**1**) was prepared from *dl-trans*-2-amino-1-cyclohexanol by the reaction with *S*-methylisothiourea as described by Bannard *et al.*[2] The optically active isomers ($\mathbf{1}_d$, $\mathbf{1}_l$) were also prepared from *d*- and *l*-2-amino-1-cyclohexanol,[3,4] respectively. Since the guanidino compounds were scarcely soluble in common organic solvents, they were converted into the salts of *p*-toluenesulfonic acid which are fairly soluble in organic solvents. These *p*-toluenesulfonates were treated with 1,1-dimethoxycyclohexane in DMF in the presence of *p*-toluenesulfonic acid. The reaction was carried out to completion[5] under reduced pressure in order to remove the generated methanol. The products ($\mathbf{5}_d$, $\mathbf{5}_l$, and **5**) obtained in good yields were confirmed to have a cyclohexylidene group by elementary analyses and PMR spectral studies. Absence of the methoxyl group shown by PMR spectra ruled out the presence of the 1-methoxycyclohexyl group which is often formed[6] in the reaction between a single hydroxyl group and 1,1-dimethoxycyclohexane. These results show that the cyclohexylidene group is bifunctionally coupled to the hydroxyl and guanidine groups, or to the amino and imino groups of the guanidine portion. The presumption is also supported[7] by the difference of the color reactions of **5** and **1** for Sakaguchi reagent; **5** was negative, whereas **1** was positive, suggesting that compound **5** is a di- or tri-*N*-substituted guanidine.

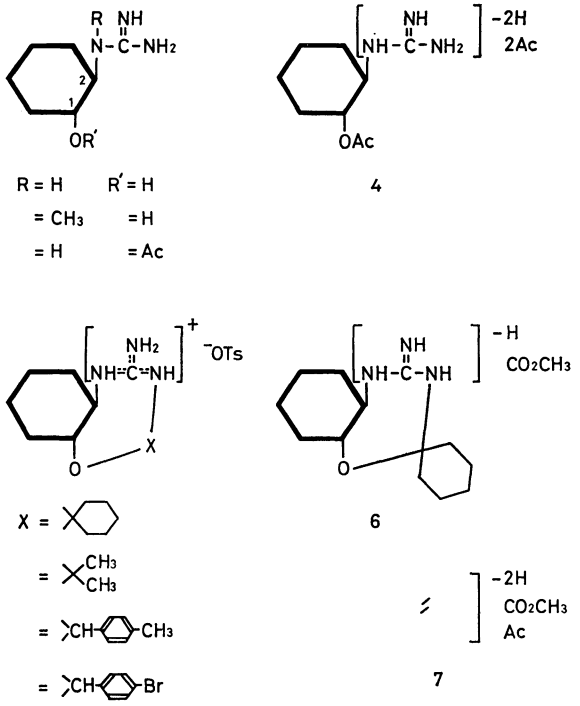
As control experiments, the *p*-toluenesulfonates of guanidinocyclohexane,[2] 2-amino-1-cyclohexanol, and 2-methylamino-1-cyclohexanol[8] were each treated with 1,1-dimethoxycyclohexane. However, no reaction took place.

In order to confirm the absence of a free hydroxyl group in **5**, **5** *p*-toluenesulfonate was treated with acetic anhydride in pyridine in the presence of triethylamine. A mixture of two products, which could not be separated by silica gel column chromatography due to their unstable nature, was obtained. However, the IR spectrum of the mixture showed no absorption peaks between 1700—1800 cm^{-1} indicating the absence of the *O*-acetyl group. On the other hand, a similar acetylation of **1** *p*-toluenesulfonate gave a triacetyl derivative (**4**), which showed an absorption peak at 1730 cm^{-1}. When the salt of **1** was acetylated with acetic anhydride in pyridine without addition of triethylamine, a mono-*O*-acetyl derivative (**3**) was formed. These results and the aforementioned result that no reaction occurs between 1-guanidinocyclohexane and 1,1-dimethoxycyclohexane indicated that **5** has a cyclohexylidene group attached to N and O, but not to N and N.

Methoxycarbonylation of **5** *p*-toluenesulfonate with methyl chloroformate and sodium hydride afforded an *N*-methoxycarbonyl derivative (**6**) in a 29% yield. Acetylation of **6** with acetic anhydride in pyridine readily gave an *N*(or *N*′**)-acetyl-*N*(or *N*′)-methoxycarbonyl derivative (**7**) as judged from their IR spectra.

$\mathbf{1}_l$ *p*-Toluenesulfonate was treated with another ketal, 2,2-dimethoxypropane in a similar manner to that for **5** to give an *N,O*-isopropylidene derivative ($\mathbf{8}_l$) in a low yield. This may be ascribed[5] to the closeness of the

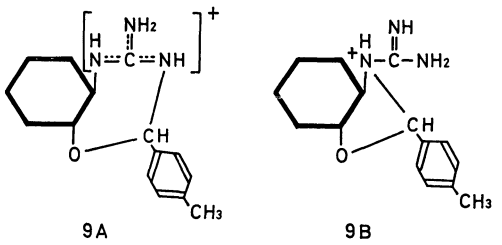
** For the sake of convenience the nitrogen directly attached to the cyclohexane ring is denoted by N and the other nitrogens by N′.



boiling points of 2,2-dimethoxypropane and methanol liberated. The structure of $\mathbf{8}_l$ was also confirmed by the acetylation of $\mathbf{8}_l$ as for **5**.

Determination of the position of nitrogen attached to the cyclohexylidene group was attempted. For this purpose, *p*-methylbenzylidene and *p*-bromobenzylidene derivatives ($\mathbf{9}_l$, $\mathbf{10}_l$)*** of $\mathbf{1}_l$ were prepared, since they were expected to have a structure similar to the cyclohexylidene derivatives (**5**, $\mathbf{5}_d$, and $\mathbf{5}_l$), and the benzylidene methine protons of the derivatives were expected to couple, in the PMR spectra, with the NH protons of the guanidine groups attached to the methine, thus revealing the position of the nitrogen in question. In the PMR spectrum of $\mathbf{9}_l$ *p*-toluenesulfonate in deuteriochloroform, the methine proton appeared as a doublet at δ 5.50. On irradiation at δ 8.34, or on deuteration, the doublet collapsed to a singlet. This suggests that the *p*-methylbenzylidene group is attached to a nitrogen bearing one hydrogen atom. However, it is still not possible to decide which one of the three nitrogens of the guanidine group is the nitrogen in question, since both **9A** and **9B** satisfy the above PMR spectral results.

Successful result was obtained from further PMR study on $\mathbf{9}_l$. Nuclear Overhauser effect was observed between the benzylidene methine proton (δ 5.50) and one of the methine protons (δ 3.2) of the cyclohexane ring. Examination of the stereomodels of **9A** and **9B** showed that only the structure **9A** can take the structure in which H-1 or H-2 of the cyclohexane ring and the methine hydrogen of the benzylidene group approach near enough for interaction. These observations suggest the structure **9A** for **9**. The IR spectra of $\mathbf{5}_d$, $\mathbf{8}_l$, $\mathbf{9}_l$, and $\mathbf{10}_l$ also supported the seven-membered structure as depicted in **9A** by the differences (75—95 cm^{-1}) in wavelength of two kinds of peaks at $\approx$1670 and $\approx$1590 cm^{-1} assignable to guanidinium ions as reported by Goto *et al.*[9]



*** By acetal formation, an asymmetric center is produced at the benzylidene methine carbon. However, in the present case, we can discuss the structures ($\mathbf{9}_l$ and $\mathbf{10}_l$) without taking the configuration of the methine into consideration.

In order to further confirm the structure, we prepared 2-(1-methylguanidino)-1-cyclohexanol (**2**, racemic), which has no hydrogen atom at N-1 of the guanidine portion, from 2-methylamino-1-cyclohexanol (racemic), and the **2** *p*-toluenesulfonate was treated with 1,1-dimethoxycyclohexane in a similar manner to that described above. However, this experiment was unsuccessful since no reaction occurred, suggesting that the 1-*N*-methyl group sterically hinders the ring formation or that the presence of the methyl group reduces the resonance stabilization of the guanidinium ion.

X-Ray crystallographic analysis[10] of $\mathbf{5}_d$ *p*-toluenesulfonate has been carried out and the result also supports the seven-membered structure.

In order to see if the above mentioned cyclohexylidenation, isopropylidenation, and benzylidenation can provide novel instances for *N,O*-protection, we first examined the stability of the protective groups in acidic or alkaline media. The compounds were fairly stable in weak acidic or weak alkaline media, but in moderately acidic or alkaline media, they were cleaved to the starting material (**1**) in a fairly short period.

In order to examine the usefulness of protection in synthesis, $\mathbf{9}_l$, selected as a model compound, was treated in pyridine with *p*-toluenesulfonyl or methanesulfonyl chlorides. The starting material ($\mathbf{9}_l$) was recovered in good yields showing that the *p*-methylbenzylidene group, as well as the guanidine portion, can resist the reaction. However, when $\mathbf{9}_l$ was treated in pyridine with acetic anhydride or benzoyl chloride at room temperature overnight, $\mathbf{9}_l$ was found to change, although the product was not identified. In the reaction of $\mathbf{9}_l$ with 50% sodium iodide in DMF at 100 °C, $\mathbf{9}_l$ resisted for 5 min, but gradually changed to **1** in a longer period. On the catalytic hydrogenation with palladium black or Raney nickel at room temperature, **5** and $\mathbf{9}_l$ were found to be stable at least for 10 h.

The *N,O*-blocked derivatives show high enhancement of the rotational value in magnitude as compared with **1**

Table 1.

Compound as *p*-toluenesulfonate	$[\alpha]_D$ (in methanol)
$\mathbf{1}_d$	−14°
$\mathbf{1}_l$	+14°
$\mathbf{5}_d$	−88°
$\mathbf{5}_l$	+84°
$\mathbf{8}_l$	+114°
$\mathbf{9}_l$	+123°
$\mathbf{10}_l$	+121°

(Table 1). This could be utilized for assigning the absolute configurations of compounds related to **1**.

Experimental

PMR spectra were recorded at 60 and 100 MHz on Hitachi R-24A and Varian XL-100 spectrometers. Thin-layer chromatography (TLC) was performed on plates of Wakogel B-5 with sulfuric acid spray for detection. For column chromatography, silica gel (Wakogel C-200) was used. When the solvent system of chloroform–methanol–28% ammonia was used with varying the ratio, the lower layers were used.

1-Guanidinocyclohexane p-*Toluenesulfonate.* A mixture of cyclohexylamine (4.75 g), *S*-methylisothiouronium sulfate (6.65 g), and water (19 ml) was treated in a similar way to that described by Bannard *et al.*,[2] and worked up as follows: After concentration to approximately two-thirds of the original volume, the reaction mixture was charged on a column of Dowex 1×2 resin (OH form, 250 ml) and developed with water. The strongly alkaline fractions were neutralized with *p*-toluenesulfonic acid and concentrated to give a solid, which was recrystallized from water to give needles, 6.65 g (44%), mp 67—69 °C. IR (KBr): 1670, 1640 cm^{-1}.

Found: C, 53.84; H, 7.35; N, 13.55; S, 10.18%. Calcd for $C_7H_{15}N_3 \cdot C_7H_7SO_3H$: C, 53.65; H, 7.40; N, 13.41; S, 10.23%.

d-, l-, *and* dl-trans-*2-Guanidino-1-cyclohexanol (**1**$_d$, **1**$_l$, and **1**)* p-*Toluenesulfonates.* These were prepared from *d*-, *l*-, and *dl-trans*-2-aminocyclohexanols and *S*-methylisothiouronium sulfate according to the method of Bannard *et al.*[2] The reaction mixture was concentrated, and the residue was boiled with methanol. The methanol solution was filtered and the filtrate was concentrated to give a solid. The solid was chromatographed on a column of Dowex resin as described above. The products, *p*-toluenesulfonates of **1**$_d$, **1**$_l$, and **1** were obtained in approximately 40% yield. They were recrystallized from methanol–ether to give plates. Each compound is soluble in water, methanol, pyridine, DMF, hot chloroform and hot acetone, but sparingly soluble in benzene, ether, and ethyl acetate. IR (KBr): 1660 and 1635 cm^{-1} (monosubstituted guanidinium[9]). **1**$_d$ *p*-Toluenesulfonate: mp 138—139 °C; $[\alpha]_D^{17}$ +6° (*c* 1, water), −14° (*c* 0.3, methanol), −6° (*c* 0.3, aqueous pyridine 1: 1). Found: C, 51.16; H, 7.06; N, 12.96; S, 9.58%. Calcd for $C_{14}H_{23}N_3O_4S$: C, 51.05; H, 7.04; N, 12.76; S, 9.73%.

1$_l$ *p*-Toluenesulfonate: mp 138—140 °C; $[\alpha]_D^{12}$ −7° (*c* 1, water), +14° (*c* 0.3, methanol), +3° (*c* 0.3, aqueous pyridine 1: 1). Found: C, 51.12; H, 6.98; N, 12.64; S, 9.64%.

1 *p*-Toluenesulfonate: mp 151—153.5 °C; $[\alpha]_D^{17}$ 0° (*c* 1, water).

dl-trans-*2-(1-Methylguanidino)-1-cyclohexanol (**2**)* p-*Toluenesulfonate.* *dl-trans*-2-Methylamino-1-cyclohexanol (2 g) prepared from cyclohexene oxide and methylamine according to the method reported[8] was dissolved in water (20 ml) and the solution was partially neutralized with *p*-toluenesulfonic acid to pH 9. Cyanamide (1.05 g) was added and the solution was heated at 60 °C for 3 h. After neutralization with *p*-toluenesulfonic acid to pH ≈7, the reaction mixture was concentrated by evaporating with ethanol and benzene. The residue was dissolved in ethanol and the solution was filtered, and evaporated. The residue was dissolved in a mixture of chloroform–methanol–28% ammonia (2: 1: 1) and the insoluble matter was removed by filtration and discarded. The filtrate was evaporated to give a residue, which was washed with water to afford a solid (2.02 g, 38%). The solid was recrystallized from chloroform–methanol–ether to give **2** *p*-toluenesulfonate as plates. It was positive to biacetyl but negative to Sakaguchi reagent. Mp 210—211 °C; IR(KBr): 1670, 1620 cm^{-1}; PMR (dimethyl-d_6 sulfoxide) δ: 2.85 (3H s, NCH_3).

Found: C, 52.38; H, 7.10; N, 12.38; S, 9.14%. Calcd for $C_8H_{17}N_3O \cdot C_7H_7SO_3H$: C, 52.46; H, 7.34; N, 12.23; S, 9.33%.

dl-trans-*1-Acetoxy-2-guanidinocyclohexane (**3**)* p-*Toluenesulfonate.* Acetic anhydride (0.17 ml) was added to a solution of **1** (305 mg) in pyridine–DMF (1: 1, 18 ml) which was then allowed to stand at room temperature for 40 h. On being subjected to TLC with chloroform–methanol–28% ammonia (1: 1: 1), the solution showed a spot at R_f 0.43. After addition of water (0.1 ml), the solution was concentrated by evaporation with toluene under reduced pressure to give a syrup. The syrup was chromatographed on a short column of silica gel with chloroform–methanol (10: 1) to give a solid of **3** *p*-toluenesulfonate, 283 mg (82%). IR (KBr): 1745 (OAc); 1680 and 1630 cm^{-1} (monosubstituted guanidinium[9]); PMR ($CDCl_3$) δ: 1.97 (3H s, OAc), 2.36 (3H s, Ts (CH_3)), 3.1—3.6 (1H m, H-2), 4.2—4.8 (1H m, H-1).

Found: C, 51.52; H, 6.68; N, 11.13; S, 8.66%. Calcd for $C_9H_{17}N_3O_2 \cdot C_7H_7SO_3H$: C, 51.74; H, 6.78; N, 11.31; S, 8.63%.

dl-trans-*1-Acetoxy-2-*[N,N′ *(or* N′,N′*)-diacetylguanidino]-cyclohexane (**4**).* Acetic anhydride (0.57 ml) and triethylamine (0.84 ml) were added to a solution of **1** *p*-toluenesulfonate (101 mg) in pyridine (2 ml), and the resulting solution was kept at room temperature for 18 h. On being subjected to TLC with benzene–ethanol (10: 1), the solution showed a spot at R_f 0.57 (**1**: R_f 0). The solution was poured into an ice-cold mixture of chloroform (25 ml) and water (25 ml) with stirring and the organic layer separated was washed with cold water, dried over magnesium sulfate and evaporated to dryness under reduced pressure. The residue was dissolved in benzene–ethanol (10: 1) and the solution was chromatographed as described above to give slightly brown crystals, 79 mg (93%), mp 66—68 °C. IR (KBr): 1730 (OAc), 1700, 1620 (s), 1560 cm^{-1}; PMR ($CDCl_3$) δ: 2.03, 2.14, and 2.18 (each 3H s, Ac), 4—4.5 (1H m, H-1?), 4.6—5.1 (1H m, H-2?), 9.15 (1H? m, broad; disappeared on deuteration).

Found: C, 54.86; H, 7.39; N, 14.65%. Calcd for $C_{13}H_{21}N_3O_4$: C, 55.11; H, 7.47; N, 14.83%.

d-, l-, *and* dl-trans-N′, O-*Cyclohexylidene-2-guanidino-1-cyclohexanol (**5**$_d$, **5**$_l$, and **5**)* p-*Toluenesulfonates.* *p*-Toluenesulfonic acid (175 mg) and 1,1-dimethoxycyclohexane (3.34 ml) were added to a solution of **1**$_d$ *p*-toluenesulfonate (1.53 g) in DMF (12 ml). The solution was heated at 50 °C for 2 h under reduced pressure (25 Torr). During this period, some solvent (≈1 ml) of the reaction mixture was distilled off. On being subjected to TLC with chloroform–methanol–28% ammonia (3: 1: 1), the solution gave a single spot at R_f 0.25. After triethylamine (3 ml) had been added, ether was gradually added until precipitation ceased. The precipitate was filtered, washed thoroughly with ether and with water to give colorless needles of **5**$_d$ *p*-toluenesulfonate (1.70 g), which were recrystallized from ethanol, 1.47 g (77%). It is soluble in aqueous pyridine (1: 1), pyridine–DMF (1: 2), chloroform–methanol (1: 1), hot pyridine, hot DMF, and hot ethanol but sparingly soluble in water even at reflux temperature. It was positive to biacetyl but negative to Sakaguchi reagent. Mp 203—205 °C, $[\alpha]_D^{21}$ −88° (*c* 0.16, methanol), $[\alpha]_D^{18}$ −110° (*c* 0.25, aqueous pyridine 1: 1). IR (KBr): 1675 and 1580 (disubstituted guanidinium[9]), 1650, 1505 cm^{-1}; PMR (pyridine-d_5–D_2O 1: 1) δ: 1.0—2.5 (≈18H), 2.30 (3H s, Ts(CH_3)), 2.5—3.2 (1H), 3.2—3.8 (1H), one AB q (4H) centered at 7.72 ($SO_2C_6H_4CH_3$).

Found: C, 58.46; H, 7.60; N, 10.43; S, 7.68%. Calcd for

$C_{13}H_{23}N_3O \cdot C_7H_7SO_3H$: C, 58.65; H, 7.63; N, 10.26; S, 7.83%.

l-Isomer, $\mathbf{5}_l$ *p*-toluenesulfonate, was similarly prepared starting from $\mathbf{1}_l$ *p*-toluenesulfonate. Mp 203—205 °C; $[\alpha]_D^{22}$ +84° (*c* 0.13, methanol); $[\alpha]_D^{16}$ +101° (*c* 0.25, aqueous pyridine 1:1). Other characteristics were the same as those of $\mathbf{5}_d$.

Found: C, 58.47; H, 7.50; N, 10.52; S, 7.95%.

dl-Isomer, **5** *p*-toluenesulfonate, was similarly prepared from **1** *p*-toluenesulfonate, $[\alpha]_D^{22}$ 0° (*c* 0.1, methanol). Mp 196—200 °C.

Found: C, 58.81; H, 7.51; N, 10.03; S, 7.52%.

dl-trans-N′, O-*Cyclohexylidene-2*-[N *(or* N′*)-methoxycarbonylguanidino*]-*1-cyclohexanol* (***6***). To a solution of **5** *p*-toluenesulfonate (501 mg) in dry pyridine–DMF (1:1, 30 ml) was added 50% oily sodium hydride (130 mg) at room temperature under a nitrogen atmosphere. After stirring for 1 h, methyl chloroformate (0.12 ml) was added and the stirring was continued for 30 min. The reaction mixture was then similarly treated as above twice with the hydride (60 mg×2) and methyl chloroformate (0.1 ml×2; added 5 min after the addition of the hydride). Chloroform (300 ml) was added with stirring to the resulting mixture, and the organic layer was washed thoroughly with water, dried over magnesium sulfate and the solvent was removed by co-evaporation with toluene. The brown syrup was chromatographed on a column of silica gel (45 g) with chloroform–ethyl acetate (2:1) as a developer. The fractions containing the main product (R_f 0.14 with the above solvent mixture) were concentrated to give a pale-yellow syrup (106 mg, 29%), which crystallized on standing. It was recrystallized from ethyl acetate to give prisms of **6**, mp 166.5—167 °C. An additional minor product (R_f 0.4, 64 mg) was also obtained. This product was presumed to be a trimethoxycarbonyl derivative as judged by its PMR spectrum, but no further study was carried out. Compound **6** was negative to biacetyl and Sakaguchi reagents. IR(KBr): 1630, 1500 cm^{-1}; PMR ($CDCl_3$) δ: 3.70 (3H s, CO_2CH_3).

Found: C, 60.83; H, 8.36; N, 13.97%. Calcd for $C_{15}H_{25}N_3O_3$: C, 60.99; H, 8.53; N, 14.23%.

dl-trans-N′, O-*Cyclohexylidene-2*-[N *(or* N′*)-acetyl*-N *(or* N′*)-methoxycarbonylguanidino*]-*1-cyclohexanol* (***7***). Acetic anhydride (0.13 ml) was added to a solution of **6** (82 mg) in pyridine (1.6 ml), and the resulting solution was kept at room temperature for 30 h. This was poured into a mixture of chloroform and saturated sodium hydrogencarbonate solution with stirring and the organic layer was washed thoroughly with water, dried over magnesium sulfate and concentrated to give a syrup, 73 mg (79%). It is soluble in most of organic solvents including hexane. IR (KBr): 1690 (shoulder), 1655, 1615 cm^{-1}; PMR ($CDCl_3$) δ: 2.25 (3H s, Ac), 3.81 (3H s, CO_2CH_3).

Found: C, 60.26; H, 8.13; N, 12.75%. Calcd for $C_{17}H_{27}N_3O_4$: C, 60.51; H, 8.07; N, 12.45%.

l-trans-*2-Guanidino*-N′,O-*isopropylidene-1-cyclohexanol* ($\mathbf{8}_l$) p-*Toluenesulfonate*. *p*-Toluenesulfonic acid (23 mg) and 2,2-dimethoxypropane (0.32 ml) were added to a solution of $\mathbf{1}_l$ *p*-toluenesulfonate (204 mg) in DMF (1.6 ml) which was heated at 50 °C for 1 h under reduced pressure (100 Torr). A few drops of the reaction mixture were taken and were poured into a mixture of triethylamine and methanol. TLC of the solution with chloroform–methanol–28% ammonia (3:1:1) showed a weak spot at R_f 0.2 ($\mathbf{8}_l$). Repeated addition of 2,2-dimethoxypropane (0.32 ml) to the above reaction mixture every 30 min and working up as above did not raise the yield of $\mathbf{8}_l$ as judged by TLC. Application of a higher reduced pressure (25 Torr) also did not improve the yield. After addition of triethylamine (0.3 ml), the reaction mixture was evaporated to give a syrup, which was chromatographed on a column of silica gel with chloroform–methanol–28% ammonia (3:2:2) to give a syrup (38 mg) containing $\mathbf{8}_l$. Repetition of the chromatography as above gave a pure solid of $\mathbf{8}_l$ (10 mg). It is soluble in methanol, benzene, chloroform, acetone, and ethyl acetate, but sparingly soluble in ether and water $[\alpha]_D^{15}$ +114° (*c* 1, methanol). IR (KBr): 1670, 1590 (disubstituted guanidinium[9]); 1510 cm^{-1}; PMR ($CDCl_3$) δ: 1.50 (6H s, $(CH_3)_2C$), 2.40 (3H s, Ts(CH_3)).

Found: C, 54.91; H, 7.25; N, 11.22; S, 8.39%. Calcd for $C_{10}H_{19}N_3O \cdot C_7H_7SO_3H$: C, 55.26; H, 7.37; N, 11.37; S, 8.68%.

l-trans-*2-Guanidino*-N′, O-p-*methylbenzylidene-1-cyclohexanol* ($\mathbf{9}_l$) p-*Toluenesulfonate*. *p*-Toluenesulfonic acid (116 mg) and *p*-tolualdehyde dimethyl acetal (2.53 ml) were added to a solution of $\mathbf{1}_l$ *p*-toluenesulfonate (1.02 g) in DMF (8 ml), and the resulting solution was heated at 50 °C for 2 h under reduced pressure (25 Torr). After addition of triethylamine (2 ml), the solution was concentrated. Addition of ether gave precipitates, which were washed successively with ether containing triethylamine and water to give a solid of $\mathbf{9}_l$ *p*-toluenesulfonate, 911 mg (66%). The solid was recrystallized from methanol–ether to give needles. It is soluble in methanol, chloroform, and pyridine, but sparingly soluble in benzene, acetone, ethyl acetate, ether, and water. It was positive to biacetyl but negative to Sakaguchi reagent. Mp 195.5—197 °C, $[\alpha]_D^{15}$ +123° (*c* 0.2, methanol). IR (KBr): 1670, 1595 (disubstituted guanidinium), 1510 cm^{-1}; PMR ($CDCl_3$) δ: 2.33 and 2.37 (each 3H s, $C\underline{H}_3C_6H_4SO_3H$ and $C\underline{H}_3C_6H_4CH=$), 3.0—3.3 (2H, broad, H-1,2), 5.50 (1H d, $CH_3C_6H_4C\underline{H}$; J= 3 Hz; collapsed to a sharp singlet on deuteration); two AB q (each 4H) centered at 7.27 and 7.30 ($J=\approx$8.5 Hz); 7.73 (1H incomplete d, NH; $J=\approx$2.5 Hz; disappeared on deuteration), 8.00 (2H s, NH; disappeared on deuteration), 8.34 (1H incomplete t, NH; J=2—3 Hz, disappeared on deuteration).

Irradiation at δ 5.50 collapsed the triplet at δ 8.34 to an incomplete doublet. Irradiation at δ 8.34 collapsed the doublets at δ 5.50 and 7.73 to a singlet, respectively.

Irradiation of the solution, after introducing nitrogen, at δ 3.15 (H-1,2) caused a 5% increase in the area of the signals at δ 5.50 ($CH_3C_6H_4C\underline{H}$).

Found: C, 61.42; H, 6.88; N, 9.56; S, 7.49%. Calcd for $C_{15}H_{21}N_3O \cdot C_7H_7SO_3H$: C, 61.23; H, 6.77; N, 9.74; S, 7.43%.

l-trans-N′, O-p-*Bromobenzylidene-2-guanidino-1-cyclohexanol* ($\mathbf{10}_l$) p-*Toluenesulfonate*. *p*-Toluenesulfonic acid (42 mg) and *p*-bromobenzaldehyde dimethyl acetal (1.4 ml) were added to a solution of $\mathbf{1}_l$ *p*-toluenesulfonate (402 mg) in DMF (3.2 ml), and the resulting solution was heated at 50 °C for 2 h under reduced pressure (25 Torr). On being subjected to TLC with chloroform–methanol–28% ammonia (1:1:1), the solution showed main spot at R_f 0.45 but $\mathbf{1}_l$ still remained. Above acetal (0.7 ml) was further added and the reaction was continued for 3 h. After addition of triethylamine (0.1 ml), the solution was processed as described for **9** to give a solid of $\mathbf{10}_l$ *p*-toluenesulfonate, 379 mg (62%). The solid was recrystallized from chloroform–methanol (1:1) to give needles. It is soluble in methanol, pyridine, DMF, but sparingly soluble in chloroform, benzene, acetone, ethyl acetate, ether, and water. It was positive to Beilstein's test for halogen. Mp 224—226 °C, $[\alpha]_D^{24}$ +121° (*c* 0.2, methanol). IR (KBr): 1670, 1595 (disubstituted guanidinium); 1520 cm^{-1}; PMR (DMSO-d_6) δ: 1.0—2.2 (8H), 2.29 (3H s, Ts(CH_3)), 5.66 (1H s, $BrC_6H_4C\underline{H}$=), 7.0—8.2 (12H, $CH_3C_6\underline{H}_4SO_3$, $BrC_6\underline{H}_4CH$=, and NH (4H), the last disappeared on deuteration).

Found: C, 51.02; H, 5.33; N, 8.20%. Calcd for $C_{14}H_{18}BrN_3O \cdot C_7H_7SO_3H$: C, 50.81; H, 5.28; N, 8.46%.

Stability of ***5*** *and* $\mathbf{9}_l$ *in Acidic and Alkaline Media*. Com-

pound $\mathbf{9}_l$ was found stable on treating its chloroform solution with saturated sodium hydrogencarbonate or 5% *p*-toluenesulfonic acid at room temperature for 5 min, as judged by TLC with chloroform–methanol–28% ammonia (1: 1: 1). Compound **5** could not be examined under the same conditions since it is scarcely soluble in chloroform. When solutions of **5** and of $\mathbf{9}_l$ in aqueous pyridine (1: 1) were kept at room temperature for 48 h, a slight conversion of $\mathbf{9}_l$ to **1** occurred, whereas **5** remained unchanged. Complete conversion of $\mathbf{9}_l$ was found to occur by keeping its aqueous methanol (1: 10) solution saturated with sodium carbonate at room temperature for 30 min, whereas, by the same treatment, less than half the amount of **5** turned to **1**. When 1.5% solutions of **5** and $\mathbf{9}_l$ in chloroform–methanol (1:1) containing 1.1 molecular equivalents (based on **5** and $\mathbf{9}_l$) of *p*-toluenesulfonic acid were kept at room temperature for 2 h, **5** completely turned to **1**, while the conversion of $\mathbf{9}_l$ to **1** was about 50%.

Reactions of $\mathbf{9}_l$ *with* p-*Toluenesulfonyl and Methanesulfonyl Chlorides.* To a solution of $\mathbf{9}_l$ *p*-toluenesulfonate (49 mg) in dry pyridine (1 ml) was added *p*-toluenesulfonyl chloride (69 mg) or methanesulfonyl chloride (45 mg) and the solution was kept at room temperature overnight. On being subjected to TLC with chloroform–methanol–28% ammonia (3: 1: 1) and chloroform-ethanol (4: 1), the solution showed a single spot (R_f 0.25 and 0.4, respectively) of $\mathbf{9}_l$. After addition of water (0.1 ml), the solution was concentrated and the resulting syrup was dissolved in chloroform. The solution was washed successively with aqueous sodium hydrogencarbonate, saturated sodium *p*-toluenesulfonate solution, water, dried over sodium sulfate and concentrated to give $\mathbf{9}_l$ *p*-toluenesulfonate, 36 mg (74%) and 36 mg (74%), respectively. Their PMR and IR spectra were indistinguishable from those of $\mathbf{9}_l$ *p*-toluenesulfonate.

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References

1) S. Umezawa, Y. Takahashi, T. Usui, and T. Tsuchiya, *J. Antibiot.*, **27**, 997 (1974).
2) R. A. B. Bannard, A. A. Casselman, W. F. Cockburn, and G. M. Brown, *Can. J. Chem.*, **36**, 1541 (1958).
3) T. Suami, S. Ogawa, and S. Umezawa, *Bull. Chem. Soc. Jpn.*, **36**, 459 (1963).
4) Absolute configurations of $\mathbf{1}_d$ and $\mathbf{1}_l$ were determined as 1S: 2S and 1R: 2R, respectively, according to S. Umezawa, T. Tsuchiya, and K. Tatsuta, *Bull. Chem. Soc. Jpn.*, **39**, 1235 (1966).
5) F. H. Bissett, M. E. Evans, and F. W. Parrish, *Carbohyd. Res.*, **5**, 184 (1967).
6) D. Ikeda, T. Suzuki, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Jpn.*, **46**, 3210 (1973).
7) J. D. Mold, J. M. Ladino, and E. J. Schantz, *J. Am. Chem. Soc.*, **75**, 6321 (1953).
8) J. B. Kay and J. B. Robinson, *J. Chem. Soc., C*, **1969**, 248.
9) T. Goto, K. Nakanishi, and M. Ohashi, *Bull. Chem. Soc. Jpn.*, **30**, 723 (1957).
10) H. Nakamura, H. Umezawa, and Y. Iitaka, the details will be described in the near future.