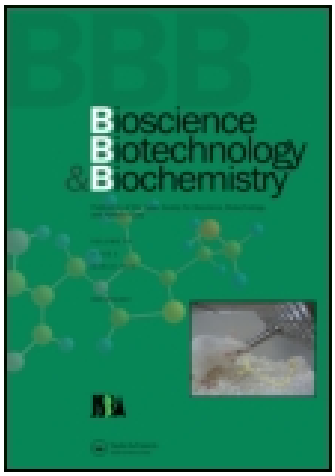

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Inhibitory Activity of 8-Azadecalin Derivatives towards 2,3-Oxidosqualene : Lanosterol Cyclases from Baker's Yeast and Pig's Liver

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The inhibitors of 2,3-oxidosqualene : lanosterol cyclase were investigated by comparative studies between pig's liver and Baker's yeast. The fundamental skeleton of the inhibitors was 8-azadecalin. To the nitrogen atom, an isoprenoid-like chain [nerylacetone (*Z*-form), geranylacetone (*E*-form) or its hydrogenated form] was attached by the reaction of reductive amination with $NaCNBH_3$. Among the three forms, the *Z*-isomer was the most potent inhibitors toward both the pig's liver and yeast cyclases. To examine the effect of carbon chain length (lipophilicity), various fatty acids (C6–C18) were appended to the 8-azadecalin derivatives. Strong inhibitory activity was observed for those compounds having carbon chains around C12. Interestingly, the amide compounds (not the carbocationic intermediate) exhibited remarkably strong inhibition toward the liver cyclase, whereas they had an insignificant effect on the yeast cyclase (about 10^2-fold less active). The yeast cyclase needed the amine functionality (carbocationic intermediate), which was prepared by using $LiAlH_4$ from the corresponding amides, to exhibit potent inhibition. We found that *N*-dodecyl-8-aza-4,4,10β-trimethyl-*trans*-decal-3β-ol (7i) was the most potent inhibitor ($IC_{50} = 1$ μM) toward the yeast cyclase amongst any known material. Kinetic studies showed that the inhibition pattern was dependent only on whether the side chains on the 8-azadecalin were linear or branched; the compounds having isoprenoid-like chains were non-competitive inhibitors, while those having linear hydrocarbon chains (amides or amines) were competitive inhibitors.

2,3-Oxidosqualene cyclase (EC 5.4.99.7) is the key enzyme for the first step of the ring cyclization reaction from the linear molecule, *i.e.*, 2,3-oxidosqualene (**1**) to form a number of sterols.[1] This cyclase catalyzes the sequential formation of four new carbon–carbon bonds leading to the tetracyclic protosterol cation (**3**), as shown in Fig. 1.[2–7] Backbone rearrangement of the protosterol cation by the cyclases gives lanosterol (**2**) in fungi and mammals, and cycloartenol or β-amyrin in photosynthetic plants.[1,7] Purification of the enzyme was achieved only a few years ago. For the yeast cyclase, one of the authors (T.H.)[8] and Corey and Matsuda[9] have reported a successful purification method, although this enzyme has been reported to be very difficult to purify.[10] With respect to both vertebrate and plant enzymes, complete purification has been achieved by Abe *et al.*[11,12] Inhibitors of the cyclases are significant for manipulating the sterol content in animals and plant cells. A very interesting phenomenon has recently been indicated that inhibitors of the cyclase can regulate HMG-CoA reductase *via* a presumed feedback mechanism involving the formation of C25-oxysterol.[13,14] The inhibitors developed so far are divided into four categories: (1) substrate mimics such as iminosqualene;[15] (2) product mimics (*e.g.*, 4,4,10β-trimethyl-*trans*-decal-3β-ol;[16,17] (3) carbocationic high-energy intermediate analogues;[18–21] and (4) irreversible inhibitors covalently bound to the enzyme.[22,23] Taton and co-workers have reported the inhibitor which can mimic the carbocationic intermediate formed during cyclization of the substrate.[24,25] This inhibitor is (±) *N*-(1,5,9-trimethyldecyl)-4α,10-dimethyl-8-aza-*trans*-decal-3β-ol (**4**) and efficiently inhibits the 2,3-oxidosqualene : lanosterol cyclase ($IC_{50} = 2$ μM) from rat's liver and the 2,3-oxidosqualene : cycloartenol cyclase ($IC_{50} = 1$ μM) from plants, although the inhibitory effect of **4** has been evaluated for a mixture of stereoisomers having two chiral centers at C1 and C5 in the hydrocarbon chain;[24] no report has yet appeared describing the stereospecific effect on the inhibitory activity. Inhibitor **4** possesses a nitrogen atom at the 8-position of the decalin skeleton. At physiological pH levels, amine groups are protonated and, therefore, the resulting ammonium derivative should present some structural and charge similarities to the postulated high-energy carbocationic intermediate as shown in Fig. 1. However, the structure-activity relationship of **4** has not been investigated in detail.[21] In order to get better knowledge on the inhibitory activity, we prepared various analogues and evaluated their inhibitory activity. Our investigation attempts to answer the following questions: (1) Is the nitrogen atom, which is responsible for the cationic species at physiological pH levels, indispensable for the potent inhibition? In other words, can a neutral species also show potent inhibition? (2) What is the role of the isoprenoid-like chain in relation to the lipophilicity or conformational flexibility for fitting to the enzyme? (3) Although inhibitor **4** has been reported to be effective toward rat's liver (mammal) and plant cyclases, is it also effective toward yeast cyclase? Comparative studies on the inhibition towards different biological species (yeast, plants, and mammals) should be carried out, because the enzyme properties are different between the mammal and yeast cyclases. For example, the molecular weights are quite different: 26 kDa for yeast,[9] and 75–78 kDa for mam-

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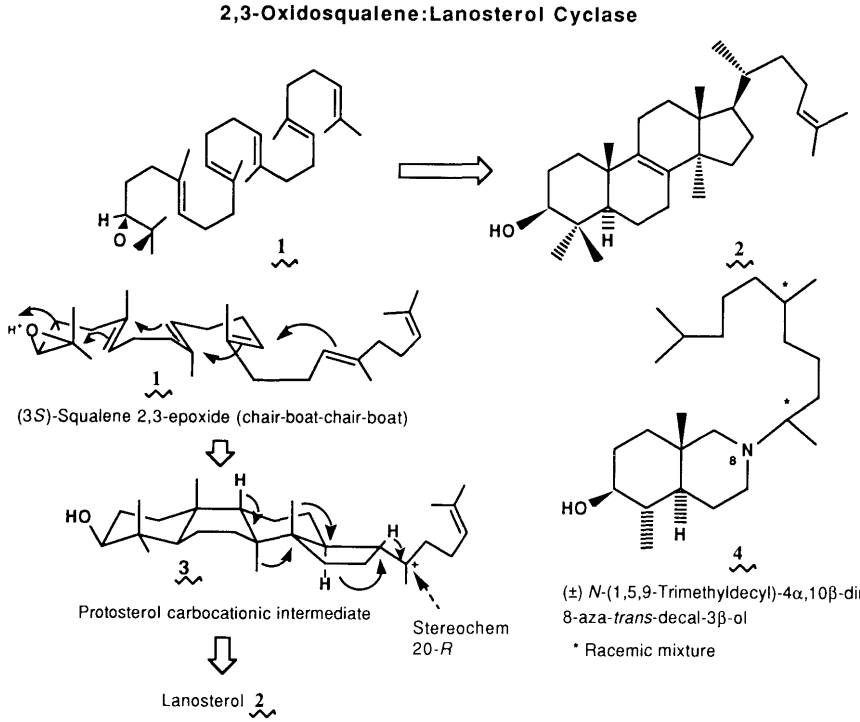

Fig. 1. Cyclization Mechanism for 2,3-Oxidosqualene (**1**) to Lanosterol (**2**) by Sterol Cyclases, and Chemical Structures of the Inhibitor (**4**) Reported by Taton *et al.* (ref. 24).

The inhibitor contained a nitrogen atom to mimic the charged center (C8) of one of the discrete high-energy intermediates formed during the cyclization process.

mals.[11] The effect of various detergents on the enzyme activity (stimulation o reduction) is also different, which is used for solubilizing the microsomal membrane proteins.[8,26] We describe here the inhibitory effect of the carbon chain length and the effect of double-bond geometry of the isoprenoid-like chains, which are connected to the nitrogen atoms of 8-azadecalin derivatives. To examine the effect of lipophilicity, fatty acyl chains having C6–C18 were connected, and the amide groups thus obtained were then reduced to give the corresponding amines. To evaluate the effect of the double-bond geometry, nerylacetone (*Z*-form) and geranylacetone (*E*-form) were used. We also report that the inhibitory activity of some compounds was relatively different toward the cyclases from yeast and mammalian sources.

Syntheses of the 8-azadecalin derivatives with isoprenoidlike chains

Figure 2 shows the four categories of 8-azadecalin derivatives (**5**, **6**, **7**, and **8**) that were examined for their inhibitory activity. Compounds in the **5** and **6** series are 4α-monomethyl-8-azadecalin derivatives, while compounds in the **7** and **8** series are 4,4-dimethyl-8-azadecalins. Those in the **5** and **7** series are 3β-ol derivatives, while those in **6** and **8** series are 3-keto derivatives. The synthetic scheme for these compounds is shown in Fig. 3. *N*-Benzyl-8-aza-4,10-dimethyldecal-4-en-3-one (**9**) was synthesized in four steps from benzylamine and methyl methacrylate according to the methods previously reported.[20,27] Subsequent Birch reduction, using Li/liq. NH_3, gave *N*-benzyl-8-aza-4α,10-dimethyl-*trans*-decal-3-one (**10a**). The 3-keto-4,4-dimethyl-azadecalin derivative (**10b**) was prepared by Birch reduction of **9** with methyl iodide. The 3-keto group was reduced with lithium aluminum hydride to give the 3β-ol (**11a** and **11b**), the stereochemistry of the hydroxyl group being confirmed as described by Rahier *et al.*[20] Compounds **11a** and **11b** were then subjected to debenzylation with H_2/5% Pd-C in acetic acid at room temperature to afford **5a** and **7a**. Nerylacetone (*Z*-isomer) was subjected to reductive amination to couple with **5a** and **7a** by using sodium cyanoborohydride. The reaction yielded **5b** and **7b** as a mixture of two diastereomers. Compounds **5c** and **7c** were prepared in the same way by using geranylacetone (*E*-isomer) from **5a** and **7a**, respectively. The ratio of the two diastereomers involved in **5b**, **5c**, **7b**, and **7c** was determined to be 65/35 by integration of the 1-methyl signal (δ_H 0.87 and 0.91, d, for N–CH–C$\underline{H}_3$) in ^{1}H-NMR spectrum and by the intensity of ^{13}C-NMR (δ_C 58.2 and 58.6 for N–C$\underline{H}$–CH_3). Catalytic hydrogenation of **5c** and **7c** with H_2/5% Pd-C yielded **5d** and **7d** having the 1,5,9-trimethyldecyl moiety, repsectively, which were also isolated as a mixture of four diastereomers (50/50 ratio at C5 and 65/35 at C1 of the side chain, which were determined from the ^{13}C-NMR signals of 5-C$\underline{H}_3$ and C1 of the side chain, respectively). The 3-keto derivatives of **6a** and **8a** were prepared by a catalytic debenzylation reaction from **10a** and **10b**, respectively. Compounds **6b**, **6c**, **6d**, **8b**, **8c**, and **8d** were synthesized from **6a** and **8a** as a mixture of diastereomers in the same manner as that just described, the ratios of the diastereomers being the same as those of compounds **5b–d**.

Syntheses of the 8-azadecalin derivatives with fatty acid chains

The *N*-benzyl-3β-ols (**11a** and **11b**) were acetylated with acetic anhydride in pyridine. Catalytic hydrogenation reactions of acetylated **11a** and **11b** gave corresponding debenzylated products **12a** and **12b**, before coupling reactions with **12a** and **12b** were carried out with *n*-fatty

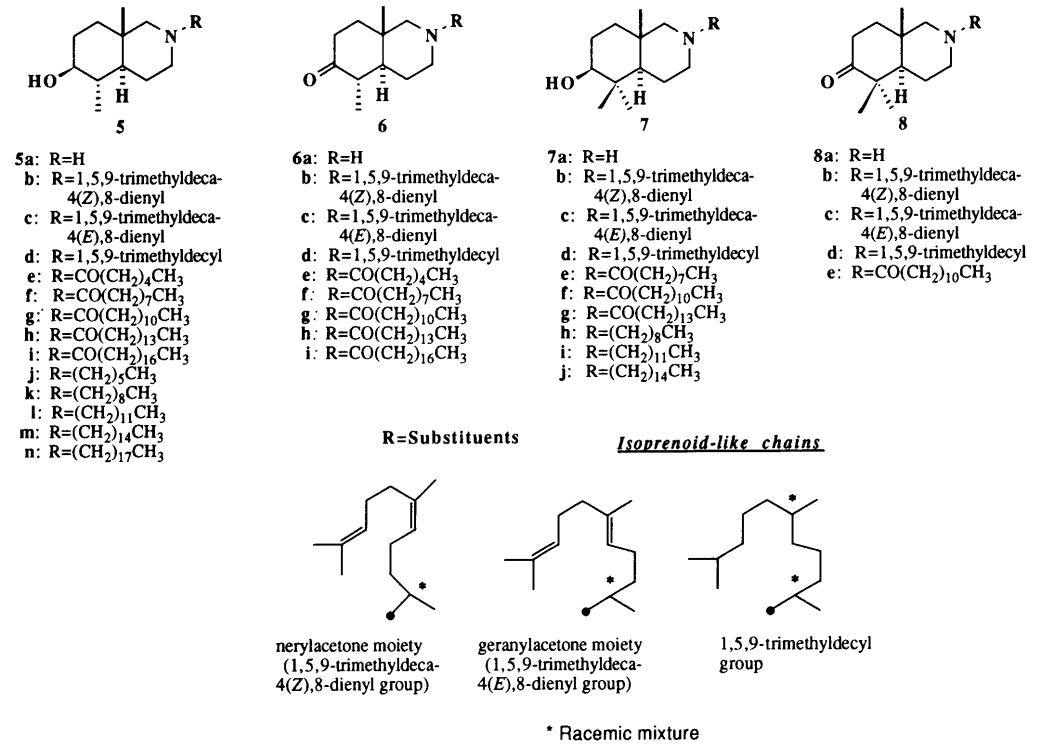

Fig. 2. Compounds Tested for the Inhibitory Activity.

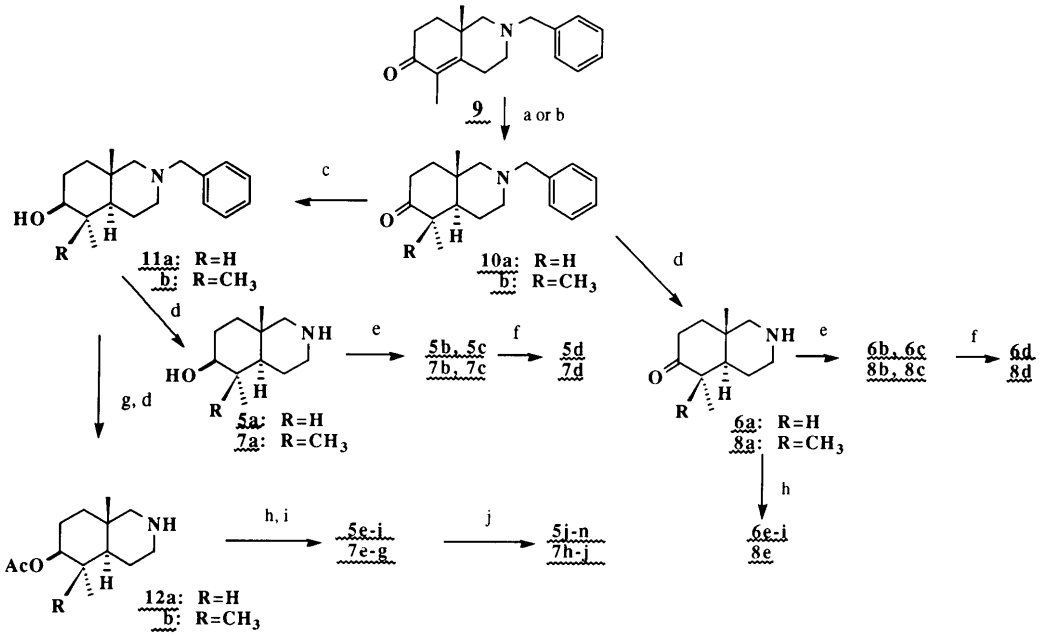

Fig. 3. Synthetic Scheme for the Tested Compounds.

Reagents: a, Li/NH_3, −40 °C; b, Li/NH_3, −40 °C, CH_3I in THF, then reflux at bp of THF; c, $LiAlH_4/Et_2O$; d, H_2/Pd-C in HOAc; e, geranylacetone or nerylacetone, $NaCNBH_3$; f, H_2/5% Pd-C; g, Ac_2O/py; h, R'COCl (fatty acid chloride, C6, C9, C12, C15 or C18), Et_3N; i, 1 N KOH/EtOH, reflux; j, $LiAlH_4/Et_2O$, reflux.

acid chlorides having different carbon chain lengths (C6, C9, C12, C15 or C18) in the presence of triethylamine as a base. The products were saponified by refluxing with 1 N KOH in ethanol to yield **5e–i** and **7e–g**. The amide functional group was then reduced with lithium aluminum hydride in ether to give the amines of **5j–n** and **7h–j**. **6e–i** and **8e** were prepared by acylating **6a** and **8a**, respectively, with various acid chlorides in the presence of triethylamine as a base.

Inhibitory Activity toward the Enzymes

The biological activity was determined by using a microsomal fraction from pig's liver or Baker's yeast. Incubation was carried out at the optimal pH levels for the enzyme activity; 6.2 for Baker's yeast[8] and 7.4 for the liver.[1] The amount of lanosterol produced increased linearly for 5 h after the incubation, the assay methods being described in detail in the Experimental section. IC_{50} values are summarized in the Table. Azadecalins themselves (**5a**, **6a**, **7a**, and **8a**) were almost inactive, although their inhibitory activity toward the liver cyclase was slightly stronger than that toward the yeast enzyme. For the effect on the liver cyclase, the β-methyl group at the 4-position

Table Inhibitory Activity of Various 8-Azadecalin Derivatives Toward the Cyclases of Pig's Liver and Baker's Yeast (IC_{50}, μM)

Compd.	Pig's Liver	Yeast	Compd.	Pig's Liver	Yeast	Compd.	Pig's Liver	Yeast	Compd.	Pig's Liver	Yeast
5a	840	>1000	**6a**	460	>1000	**7a**	180	>1000	**8a**	200	>1000
b	3	4	**b**	130	650	**b**	11 (15*)	3 (5*)	**b**	90	75
c	18, 4[a]	31	**c**	250	>1000	**c**	20	13	**c**	90	160
d	10(9*), 2[b], 1[a]	25 (25*)	**d**	10	72	**d**	13	7	**d**	130	110
e	80	220	**e**	180	>1000	**e**	7	200	**e**	18	>1000
f	1.2	130	**f**	120	500	**f**	3 (2.2**)	120			
g	1.2 (0.9**), 0.11[b]	100 (91**)	**g**	8	250	**g**	11	150			
h	3	250	**h**	130	340	**h**	4	3			
i	28	680	**i**	190	400	**i**	4 (3.3**)	1 (1.1**)			
j	>1000	>1000				**j**	22	18			
k	5.5	75									
l	9, 0.55[b]	18 (16**)									
m	200	70									
n	500	420									

Parentheses indicate the K_i value (μM), which are close to those of IC_{50}. Symbols of * and ** indicate noncompetitive and competitive inhibition, respectively. a) and b) show IC_{50} for oxidosqualene : cycloartenol cyclase from maize seedling and oxidosqualene : lanosterol cyclase from rat's liver, respectively. Values of a) and b) were cited from refs. 21 and 29.

of the azadecalins takes effect on the fitting of the enzyme, because the inhibitory activity of both **7a** and **8a** were higher than that of **5a** and **6a**. When the lipophilic side chains (isoprenoid-like chains) were attached to the azadecalin skeleton, the inhibitory activity was dramatically increased as shown in the Table (*e.g.*, compare **5a** with **5d**). The effect of geometrical isomers (*E*, *Z*) on the inhibitory activity was also examined. In the case of 4α-methylazadecalin, the *Z*-isomer (**5b**) was the most active toward both the yeast ($IC_{50}=4\,\mu$M) and the liver cyclase ($IC_{50}=3\,\mu$M), when compared to the *E*-isomer (**5c**, $IC_{50}=31$ or $18\,\mu$M) and the saturated hydrocarbon (**5d**, 25 or $10\,\mu$M). This was also true of the 4,4-dimethylazadecalin derivatives (**7b**, **7c**, and **7d**). Taton *et al.* have reported the IC_{50} values for **5c** and **5d** for the rat's liver cyclase and the plant cyclase.[21,24] However, the inhibitory activity of related compounds **5b** and **7b–d** has not previously been reported,[28] nor has the inhibition mechanism been described for these related compounds having isoprenoid-like chains.[20,21,24,28] From Lineweaver–Burk plots (data not shown), **5d** and **7b** were determined to be non-competitive inhibitors (see the Table), the values of K_i being close to those of IC_{50}. The 3-keto azadecalins coupled with the isoprenoid-like chains exhibited lower activity than the corresponding 3-OH compounds, indicating that the hydroxyl group at the 3-position was responsible for the interaction with the enzymes, probably *via* hydrogen bonding.

Next, we examined the effect of lipophilicity of the side chains on the inhibitory activity. The fatty acyl chains having C6–C18 were coupled with the 8-azadecalins. With regard to the **5** amide series, C9–C15 (**5f**, **5g**, and **5h**) were more effective for inhibiting the pig liver cyclase, when compared to C6 or C18 (**5e** or **5i**). For the amine compounds of the **5** series, C9–C12 (**5k** and **5l**) were the most active among C6–C18 (**5j–n**). In the case of the 4,4-dimethylazadecalinols (the **7** series), strong inhibitory activity was found in C9–C15 for the amides (**7e**, **f**, and **g**) and in C9–C12 for the amines (**7h** and **7i**) as was also observed with the **5** series. For the 3-ketoazadecalins (the **6** series), C12 (**6g**) showed the prominently strong activity among C6–C18 (**6e–i**). Compound **8e**, having C12 length, also showed strong activity toward the liver cyclase. On the other hand, for the yeast cyclase, carbon number C12 resulted in the highest inhibitory activity amongst compounds in the **5**, **6**, and **7** series, regardless of the amide or amine functionality. Of all the compounds prepared by us, it seems plausible that the carbon chain length for best fitting to the two cyclase enzymes is around C12.

Very interestingly, the amide compounds strongly inhibited the pig's liver cyclase, but had an insignificant effect on the yeast cyclase (see the Table); compounds **5f**, **5g**, and **5h** were *ca.* 10^2-fold less active toward the yeast cyclase than toward the pig's liver enzyme. The amide compounds of the **5**, **6**, **7**, and **8** series are not a cationic species, but a neutral form at physiological pH levels. This finding suggests that, for mammalian cyclases, the protonated ammonium cation was not necessary for inhibition, while in case of the yeast cyclase, the carbocationic species was strictly required, as exemplified in the comparison between **7e–g** and **7h–j** (Table). For the yeast cyclase, the 4,4-dimethylazadecalin derivatives (**7h–j**) showed considerably enhanced inhibition, when compared to the corresponding 4α-monomethylazadecalin derivatives (**5k–m**). This fact strongly suggests that the 4β-methyl group played a significant role in the inhibition toward the yeast cyclase. **7i** exhibited the most potent inhibition ($IC_{50}=1\,\mu$M) for the cyclase from *Saccharomyces cerevisiae* amongst any known compounds.[28] The inhibitory activity of each of the 3-keto azadecalin compounds (**6e–i** and **8e**) was relatively weak, when compared to that of each of the corresponding 3β-OH compounds (**5e–i** and **7f**), suggesting that the β-hydroxyl group interacted in part with the enzymes. Compound **5g** has very recently been reported to be a potent inhibitor of rat liver cyclase.[29] However, differences in the biological species, the effect of carbon chain length and related compounds have not previously been reported. It is noteworthy that **5f–h** (amide compounds) were active only toward the pig cyclase, and ineffective toward the yeast variety; this biological selectivety has not been reported by any other workers. We examined whether or not the inhibition mechanism would be different between the mammalian and the yeast cyclases.

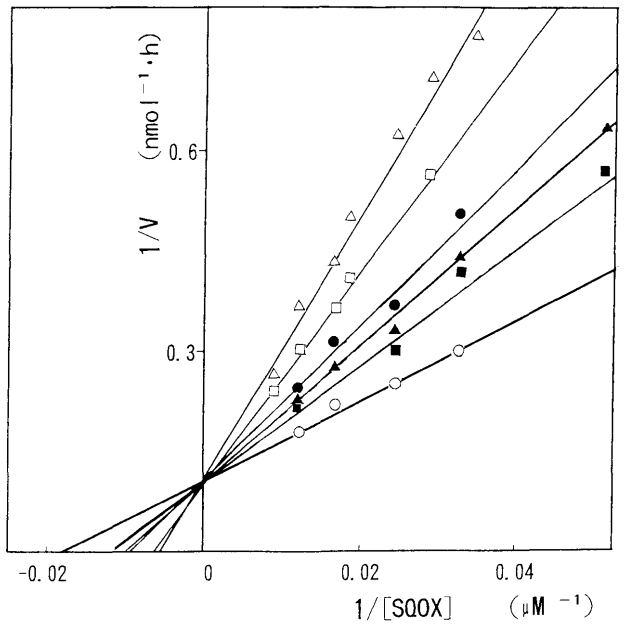

Fig. 4. Lineweaver–Burk Plots of the Inhibition by Compounds **7f** and **7i** of the Pig's Liver Cyclase.

Inhibitor concentrations: ○ 0 μM; ■ 1 μM; ▲ 3 μM; ● 4 μM; □ 3 μM; △ 5 μM. Symbols □ and △ represent compound **7f** (amide), while ■, ▲, and ● denote compound **7i** (amine). Abbreviations SQOX and V mean the substrate concentration of the *S*-isomer and the amount of lanosterol produced in 1 h during the incubation, respectively. The K_m value for the pig's liver cyclase was determined to be 55 μM.

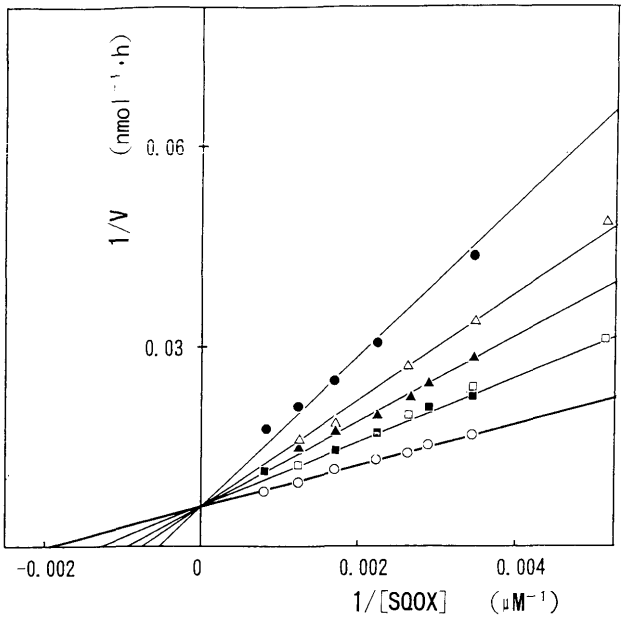

Fig. 5. Lineweaver–Burk Plots of the Inhibition by Compounds **5g** and **7i** of the Baker's Yeast Cyclase.

Inhibitor concentration: ○ 0 μM; ■ 1 μM; ▲ 3 μM; ● 4 μM; □ 50 μM; △ 100 μM. Symbols □ and △ represent compound **5g** (amide), while ■, ▲, and ● denote compound **7i** (amine). The other symbols are the same as those defined in Fig. 4. The K_m value of the Baker's yeast was determined to be 518 μM.

The kinetic studies (Figs. 4 and 5) demonstrated, however, that **5g**, **5l**, **7f**, and **7i** were all competitive inhibitors towards both the yeast and pig's liver cyclases. This also suggests that the inhibition mechanism for the amides and amines is the same, irrespective of any functional differences. The selective inhibitors among the amide compounds, *e.g.*, **5f–h** and **7e–g**, which were effective only toward the mammalian species, might be a promising tool for discriminating between cyclase enzymes from different biological sources.

In summary, we found potent inhibitors (amide compounds) which are selectively efficient toward mammalian cyclases, and also found **7i** to be one of the most potent inhibitors toward yeast cyclase amongst any known materials. We also show that **5d**, having the isoprenoid-like chain, was a non-competitive inhibitor toward both the mammalian and yeast cyclases, while **5l**, having a normal hydrocarbon chain, was competitive (Table). This suggests that the branched chain has affinity for different domain(s) from the substrate-binging site(s), judging from the inhibition mechanism being non-competitive. The substrate, 2,3-oxidosqualene, has the *E*-geometry. Therefore, the *E*-isomer with the isoprenoid-like chain has been considered to be more active than the *Z*-isomer owing to its structural similarity to the substrate. However, our experimental results were otherwise; the *Z*-isomer was more active toward the two enzymes from the yeast and mammalian sources (compare **5b** with **5c** and **7b** with **7c**). This finding may further support the idea, obtained from the kinetic study (non-competitive), that the binding site of the isoprenoid chain to the enzyme is different from that of the substrate. The reason remains unclear why the amide compounds (**5f–h** and **7e–g**) showed selective inhibition toward pig's liver cyclase. Wannamaker *et al.* have not fully discussed why **5g** was a potent inhibitor toward rat's liver cyclase,[29] irrespective of the lack of the amine functionality that is required for the cationic species. The amide compounds, as well as the corresponding amines, showed competitive inhibition with respect to the substrate, regardless of the different biological species (Figs. 4 and 5). The amide group of azadecalins may interact in part with the specific amino acid(s), this possibly only being involved with the liver cyclase. However, this interpretation is not likely, because the activity of **6f** and **6h** was significantly lower than that of **5f** and **5h**, although the inhibitory activity of **5g** and **6g** was not very different. If there is strong interaction between the amide group and the specific amino acid(s) of the liver cyclase, the activities of **6f** and **6h** would not be dramatically less. Another interpretation is that the conformation of the azadecalinol with the amide group may be tailored to the liver cyclase. NMR data for the amide compounds (**5e–i** and **7e–g**) indicates a planar B ring due to the hindered rotation around the N–C (O) bond of the amide group, as was also pointed out for **5g** by Wannamaker *et al.*[29] The mammalian enzymes may have a greater affinity for the planar conformation of the azadecalinol than the yeast cyclase. The potent activity of the amide compounds may be brought about as a consequence of the planar conformation, the 3β-hydroxyl group and the hydrophobicity of the side chains. Further studies will be necessary for a better-understanding of the inhibition mechanism and of the enzymic properties from different biological sources.

Experimental

Exzyme preparation.

Yeast cyclase. The crude enzymes were prepared according to the methods described in refs. 8 and 30. Baker's yeast (Saf-instant, S.I. Lesaffre, France) was used as the enzyme source, 60 g of freeze-dried yeast suspended in 300 ml of a 0.1 M phosphate buffer (pH 6.8) being stirred at 4°C to obtain a homogeneous mixture. The suspension was then sonicated by a cell disruptor, using a large titanium tip for 60 min in the continuous sonication mode, the inside temperature being kept below 10°C with a

bath cooler. This protocol gave the best conversion ratio from substrate to product. The disrupted cell suspension was centrifuged at 17.000 × *g* to remove debris, the supernatant being used for the usual assay. The protein content was usually in the range of 4–7 mg/ml according to the Lowry method. The supernatant was further centrifuged at 100,000 × *g* by a preparative ultracentrifuge to separate the microsomal fraction from the soluble fraction, the microsomal fraction being used for determining the inhibition constant (K_i).

Pig's liver cyclase.[26] Fifty grams of pig's liver was homogenized at 4 C in a Waring blender with 90 ml of a 0.1 M Tris–HCl buffer solution (pH 7.4) containing 1 mM EDTA and 40 mg/ml of phenylmethylsulfonyl fluoride, and the mixture then centrifuged for 10 min at 17,000 × *g*. The resulting supernatant was further centrifuged at 100,000 × *g* for 90 min. the resulting microsomal pellets were resuspended and homogenized in the original quantity of Tris–HCl buffer, using a Potter Elvehjem homogenizer. The protein amount was usually in the range of 20–25 mg/ml, and the suspension was used for the enzyme assay. When stored at −20 C, the enzymes thus prepared from yeast and pig's liver were stable for at least 1 month.

Assay methods. The conversion of the substrate to lanosterol was carried out by emulsifying [*R*, *S*]-squalene oxide (1 mg for yeast; 0.1 mg for liver) with Triton X-100 (14 mg for yeast; 8.4 mg for liver) in 2.5 ml (a 0.1 M K-phosphate buffer at pH 6.2) for yeast for 2.8 ml (0.1 M Tris–HCl at pH 7.4) for liver, and then 0.5 ml for yeast or 0.2 ml for liver of the enzyme fraction was added. The solution was flushed with nitrogen and then incubated for 5–8 h at 37 C under anaerobic conditions, the final volume being 3.0 ml. The substrate concentrations for the *S*-configuration (39.1 and 391 μM for liver and yeast, respectively) were close to the K_m values. To terminate the incubation, 4 ml of a 15% methanolic KOH solution was added, and the incubation tube was heated to 70 C for 20 min to saponify the reaction mixture, before extracting with 1.5 ml of *n*-hexane. The product, lanosterol, was analyzed by GLC (Shimadzu GC-8A), the GLC conditions being as follows: sample size, 5 μl; column, OV-1 3%; injection temp., 300 C; column temp., 280 C; carrier gas, 40 ml/min. The conversion quantity from the *S*-oxidosqualene to lanosterol was for the liver cyclase, from 117.3 nmol of the substrate to 20 nmol of the product after the incubation for 3 h, and for the yeast cyclase, from 1173 to 350 nmol after 5 h. IC_{50} values were evaluated after 5 h of incubation, while the inhibition constants (K_i) were determined after incubating for 5 and 3 h for the yeast and for liver cyclases, respectively.

Materials. NMR spectra were obtained with a Varian Gemini 200H instrument. ^{1}H- and ^{13}C-NMR spectra for all the compounds were measured in $CDCl_3$, the chemical shifts being given in ppm relative to the ^{1}H signal (7.24 ppm) and the central signal for $CDCl_3$ (77.0 ppm). The signals were assigned with the aid of DEPT, H–H and C–H COSY pulse sequences or by spin decoupling. EIMS spectra, including HREIMS, were measured with a JEOL SX102 instrument (direct inlet system, 70 eV). The purity of all the compounds was checked by a GLC analyses in an OV-17 column.

2,3-[*R*,*S*]-Oxidosqualene was synthesized from squalene by the method of Nadeau *et al.*[31]

N-Benzyl-8-aza-4,10-dimethyl-decal-4-en-3-one (**9**).[20,27] The synthetic method was accoding to refs. 20 and 27. NMR δ_H: see ref. 20; δ_C: 198.8 (s), 160.0 (s), 138.7 (s), 128.6 (d), 128.5 (s), 128.2 (d), 126.9 (d), 66.8 (t), 62.5 (t), 54.0 (t), 37.1 (s), 34.3 (t), 33.5 (t), 28.2 (t), 23.0 (q), 10.6 (q). *Anal.* Found: C, 80.58; H, 8.50; N, 4.96%. Calcd. for $C_{18}H_{23}NO$: C, 80.26; H, 8.61; N, 5.20%.

N-Benzyl-8-aza-4α,10-dimethyl-trans-decal-3-one (**10**).[20] A solution of **9** (11.3 g, 42 mmol) and dry *t*-BuOH (3.0 g) in dry THF was added at −40 C to a suspension of Li (4.5 g) in dry NH_3 (840 ml). The reaction was allowed to proceed for 3 h, before the excess Li was destroyed by adding bromobenzene until the reaction mixture had become clear. Saturated aq. NH_4Cl was then added, and the product was extracted with Et_2O. The ethereal extract was washed with water and dried over Na_2SO_4, and then subjected to column chromatography over SiO_2 with hexane–EtOAc = 100 : 5 (saturated with aq. NH_3). The yield was 7.26 g (63.8%). NMR δ_H: see ref. 20; δ_C: 212.6 (s), 139.0 (s), 128.6 (d), 128.1 (d), 126.7 (d), 66.4 (t), 62.7 (t), 54.4 (t), 49.9 (d), 45.0 (d), 38.3 (t), 37.7 (t), 34.4 (s), 26.4 (t), 17.2 (q), 10.9 (q). MS *m/z*: 271 (M^+). *Anal.* Found: C, 80.80; H, 9.49; N, 4.95%. Calcd. for $C_{18}H_{25}NO$: C, 79.66; H, 9.28; N, 5.16%.

N-Benzyl-8-aza-4,4,10-trimethyl-trans-decal-3-one (**10b**).[20] A solution of **9** (500 mg) in dry THF (10 ml) wa added at −40 C dropwise to a suspension of Li in NH_3 (50 ml). The reaction was allowed to continue for 2 h, 3 g of CH_3I in THF (40 ml) was added, and the solution was refluxed at the bp of THF for 2 h, before being allowed to stand overnight. The reaction mixture was next poured into water and extracted with Et_2O. The ethereal layer was washed with brine and dried with Na_2SO_4, the column chromatography (SiO_2) with hexane–AcOEt = 10 : 1 (saturated with aq. NH_3) affording 240 mg of the product (45.6% yield). NMR δ_H: see ref. 20; δ_C: 216.5 (s), 139.0 (s), 128.6 (d), 128.1 (d), 126.7 (d), 69.2 (t), 62.7 (t), 54.9 (t), 52.1 (d), 47.3 (s), 37.1 (t), 34.5 (t), 25.2 (q), 22.9 (t), 21.3 (q), 19.1 (q). MS *m/z*: 285 (M^+).

N-Benzyl-8-aza-4α,10-dimethyl-trans-decal-3β-ol (**11a**).[20] **10a** (3.12 g, 11.5 mmol) was dissolved in 56 ml of ether at −5 C. To the solution was added a suspension of $LiAlH_4$ (0.635 g, 17 mmol) in ether (111 ml), and the mixture was stirred for 4 h at room temperature, and the reaction was stopped by adding EtOAc saturated with water. The residue was taken up with ether, dried, and then crystallized from hexane to give a pure product (2.1 g, 66.5%). NMR δ_H: see ref. 20; δ_C: 139.3, 128.6, 127.9, 126.6, 67.4, 62.9, 55.1, 47.9, 38.9, 36.6, 34.3, 30.7, 25.1, 17.9, 14.7. MS m/z: 273 (M^+). *Anal.* Found: C, 79.28; H, 9.76; N, 4.96%. Calcd. for $C_{18}H_{27}NO$: C, 79.07; H, 9.95; N, 5.12%.

N-Benzyl-8-aza-4,4,10-trimethyl-trans-decal-3β-ol (**11b**).[20] This was prepared from **10b** in the same manner as that described for **11a**. NMR δ_H: see ref. 20; δ_C: 139.2 (s), 128.7 (d), 128.0 (d), 126.7 (d), 79.2 (d), 70.4 (t), 62.9 (t), 55.6 (t), 51.6 (d), 38.3 (s), 36.6 (t), 34.7 (s), 27.6 (q), 27.3 (t), 22.0 (q), 20.0 (q), MS *m/z*: 287 (M^+). *Anal.* Calcd: C, 79.28; H, 10.48; N, 4.96%. Calcd. for $C_{19}H_{29}NO$: C, 79.39; H, 10.17; N, 4.87%.

8-Aza-4α,10-dimethyl-trans-decal-3β-ol (**5a**)[20] **11a** (1.80 g, 6.57 mmol) was dissolved in 40 ml of acetic acid. To the solution was added 0.8 g of 5% Pd-C, and the mixture stirred in an atmosphere of H_2 gas. After 10 h, the reaction mixture was filtered to remove Pd-carbon, and concentrated *in vacuo* to dryness. To the residue was added water (5 ml), the pH being adjusted to 10 with Na_2CO_3, and the solution concentrated again to dryness under reduced pressure. The residue was refluxed with $CHCl_3$, and filtration and evaporation of the solution gave the pure product (1.14 g, 95%). NMR δ_H: see ref. 20; δ_C: 76.2 (C3 of decalin), 59.9 (C9), 47.9 (C7), 47.3 (C5), 37.1 (C4), 36.4 (C1), 33.6 (C10), 30.7 (C2), 25.3 (C6), 16.4 (C10, CH_3), 14.5 (C4, CH_3). HREIMS *m/z* (M^+): 186.1619; calcd. for $C_{11}H_{21}NO$: 183.1623.

8-Aza-4,4,10-trimethyl-trans-decal-3β-ol (**7a**). The preparation method for **7a** from **11b** was analogous to the debenzylation reaction of **11a**. HREIMS *m/z* (M^+): 197.1775; calcd. for $C_{12}H_{23}NO$: 197.1780.

8-Aza-4α,10-dimethyl-trans-decal-3-one (**6a**). NMR δ_H: 3.14 (1H, br d, J = 11 Hz), 2.1–2.8 (6H, m), 1.1–1.7 (6H, m), 1.17 (3H, s), 0.92 (3H, d, J = 6.5 Hz); δ_C: 212.0 (s), 59.0 (t, C9 of decalin), 49.9 (d, C5), 46.4 (t, C7), 45.1 (d, C4), 38.2 (t, C1), 37.7 (t, C2), 33.8 (s, C10), 26.2 (t, C6), 15.9 (q, C10 CH_3), 10.8 (q, C4 CH_3).

8-Aza-4,4,10-trimethyl-trans-decal-3-one (**8a**) NMR δ_H: 3.20 (1H, br d, J = 10 Hz), 2.2–2.8 (5H, m), 1.0–1.7 (6H, m), 1.18 (3H, s), 1.03 (3H, s), 0.99 (3H, s); δ_C: 215.9 (s), 61.9 (t, C9 of decalin), 52.0 (d, C5), 47.4 (s, C4), 46.9 (t, C7), 37.0 (t, C1), 34.5 (t, C2), 34.0 (s, C10), 25.0 (q, C4ax., CH_3), 22.9 (t, C6), 21.3 (q, C4eq., CH_3), 17.9 (q, C10 CH_3). The abbreviations ax. and eq. mean axial and equatorial, respectively.

8-Aza-4α,10-dimethyl-trans-decal-3β-ol-acetate (**12a**). Compound **11a** was acetylated with Ac_2O/py by a standard method, and debenzylation was carried out by using H_2/Pd-C (5%) in HOAc at room temperature. NMR δ_H: 4.36 (1H, ddd, J = 10, 10, 5 Hz), 3.08 (1H, br d, J = 12 Hz), 2.41–2.60 (2H, m), 2.21 (1H, d, J = 12 Hz), 1.99 (3H, s), 0.8–1.9 (9H, m), 0.93 (3H, s), 0.74 (3H, d, J = 6.4 Hz); δ_C: 170.7 (s), 78.6 (d), 60.1 (t), 48.0 (d), 47.4 (t), 35.9 (2 × C; d and t), 33.5 (s), 26.6 (t), 25.2 (t), 21.2 (q), 16.4 (q), 14.4 (q), *Anal.* Found: C, 69.61; H, 10.41; N, 6.01%. Calcd. for $C_{13}H_{23}NO_2$: C, 69.29; H, 10.29; N, 6.22%.

8-Aza-4,4,10-trimethyl-trans-decal-3β-ol-acetate (**12b**). NMR δ_H: 4.47 (1H, dd, J = 10, 6 Hz), 3.14 (1H, br d, J = 12 Hz), 2.4–2.6 (2H, m), 2.20 (1H, d, J = 12 Hz), 2.02 (3H, s), 1.95 (1H, br s), 1.65 (2H, m), 0.95–1.47 (5H, m), 1.00 (3H, s), 0.81 (3H, s), 0.79 (3H, s); δ_C: 170.8 (s), 80.7 (d), 63.5 (t), 51.7 (d), 47.9 (t), 37.3 (s), 36.1 (t), 34.3 (s), 27.3 (q), 23.6 (t), 22.2 (t), 21.2 (q), 18.8 (q), 16.2 (q).

General Procedure for the syntheses of 8-azadecalin coupled with isoprenoid-like chains. Geranylacetone and nerylacetone were obtained from Aldrich Co. To a solutions of an 8-azadecalin derivative (**5a**, **6a**, **7a**, or **8a**; 0.82 mmol) and the isoprenoid-like compound (nerylacetone or geranylacetone, 0.88 mmol) dissolved in dry MeOH (3 ml) was added 42 mg of $NaBH_3CN$, and the mixture was stirred for 72 h. The reaction mixture was dried, taken up with hexane, and subjected to column chromatography (SiO_2). Elution with hexane–EtOAc = 100 : 5 (saturated with aq. NH_3) gave

pure products (**5bc**, **6bc**, **7bc**, and **8bc**), the yield of the reductive amination usually being 36–39%. The hydrogenated products (**5d**, **6d**, **7d**, and **8d**) were obtained from **5c**, **6c**, **7c**, or **8c** by catalytic hydrogenation, using H_2/PtO_2. A suspension of each *E*-compound (0.11 mmol) and PtO_2 (5 mg) in EtOH (3 ml) was stirred for 48 h in an atmosphere of H_2. The product was purified in a short column over SiO_2, the yield of the hydrogenation reaction usually being 92–95%.

N-(1,5,9-Trimethyldeca-4(Z),8-dienyl)-8-aza-4α,10-dimethyl-trans-decal-3β-ol (**5b**). NMR of the one major diastereomer: δ_H 5.09 (2H, br t, $J=6.7$ Hz, olefinic protons), 3.08 (3ax., ddd, $J=11$, 10, 5 Hz), 2.69 (7eq., ddd, $J=11$, 2, 2 Hz), 2.2–2.4 (N–C*H*– & 9eq., m), around 2.0 (6H, m, CH_2 of side chains), 1.82 (7ax., ddd, $J=11$, 11, 3.5 Hz), 1.66 (CH_3 on the olefin), 1.58 ($2\times CH_3$ on the olefin), 1.4–1.6 (6eq., & N–CH–CH_2–, AB spin system), 1.1–1.4 (9ax., & N–CH–CH_2–, AB), 0.97 (10ax., CH_3, s), 0.91 (N–CH–CH_3, d, $J=6.5$ Hz), 0.83 (4eq., CH_3, d, $J=6.6$ Hz), 0.61 (5ax., ddd, $J=12$, 11, 3.6 Hz). The other signals of the decalin were masked in the range of 0.8–1.7 ppm (aliphatic signals of the isoprenoid chain), and the ratio of the two diastereomers being determined by integrating the methyl signal of N–CH–CH_3 (0.87, d, $J=6.7$ Hz for the alternative diastereomer) to be about 65 : 35. NMR: δ_C 134.9 (s), 131.4 (s), 125.6 (d), 124.4 (d), 76.9 (d, C3 of decalin), 59.1 (t, C9), 58.2 (d, N–*C*H–CH_3), 53.0 (t, C7), 48.4 (d, C5), 39.0 (d, C4), 36.8 (t, C1), 34.7 (t), 34.4 (s, C10), 33.9 (t), 31.9 (t), 30.7 (t, C2), 26.7 (t, C6), 25.7 (q), 25.5 (t), 25.0 (t), 23.4 (q), 17.9 (C10 CH_3, q), 17.6 (q), 14.6 (C4 CH_3, q), 12.6 (q); 58.6 (d) for N–*C*H–CH_3 for the alternative diastereomer. The ratio of each intensity (δ_C 58.2/58.6) also indicated the ratio of 65/35 for two diastereomers. HREIMS *m/z* (M^+): 361.3351; calcd. for $C_{24}H_{43}NO$: 361.3345. *Anal.* Found: C, 79.61; H, 12.11; N, 3.78%. Calcd.: C, 79.72; H, 11.99; N, 3.87%.

N-(1,5,9-Trimethyldeca-4(E),8-dienyl)-8-aza-4α,10-dimethyl-trans-decal-3β-ol (**5c**). NMR of the one major diastereomer: δ_H 5.08 (2H, t-like, $J=6.7$ Hz, olefinic protons), 3.08 (3ax., ddd, $J=12$, 9, 5 Hz), 2.70 (7eq., ddd, $J=13$, 2, 2 Hz), 2.20–2.29 (N–C*H*– & 9eq., m), 1.98 (6H, m, CH_2 of side chains), 1.78 (7ax., ddd, $J=11$, 11, 3.5 Hz), 1.65 (CH_3 on the olefin), 1.58 ($2\times CH_3$ on the olefin), 1.4–1.6 (6eq., & N–CH–CH_2–, AB), 1.1–1.4 (9ax., & N–CH–CH_2–, AB), 0.98 (10ax., CH_3, s), 0.91 (N–CH–CH_3, d, $J=6.5$ Hz), 0.83 (4eq., CH_3, d, $J=6.6$. Hz), 0.61 (5ax., ddd, $J=12$, 11, 3.6 Hz). The ratio of the two diastereomers were determined by integrating the methyl signal of *N*–CH–CH_3 (0.87, d, $J=6.7$ Hz for the alternative diastereomer) to be about 65 : 35. NMR δ_C: 134.1 (s), 130.6 (s), 124.1 (d), 123.7 (d), 76.2 (d, C3 of decalin), 58.5 (d, C9), 57.4 (d, N–*C*H–CH_3), 52.3 (t, C7), 47.8 (d, C5), 39.1 (t), 38.4 (d, C4), 36.2 (t, C1), 33.8 (s, C10), 33.7 (t), 33.3 (t), 30.1 (t, C2), 26.1 (t, C6), 25.1 (q), 24.8 (t), 24.5 (t), 17.3 (C10 CH_3, q), 15.3 (q), 14.0 (C4 CH_3, q), 12.1 (q); 57.7 (d) for N–*C*H–CH_3 for the other diastereomer. HREIMS *m/z* (M^+): 361.3353; calcd. for $C_{24}H_{43}NO$: 361.3345. *Anal.* Found: C, 79.66; H, 12.20; N, 3.46%. Calcd.: C, 79.72; H, 11.99; N, 3.87%.

N-(1,5,9-Trimethyldecyl)-4α,10-dimethyl-8-aza-trans-decal-3β-ol (**5d**). HREIMS *m/z* (M^+): 365.3662; calcd. for $C_{24}H_{47}NO$: 365.3658. The diastereomers involved in the side chains were found in the ^{13}C-NMR: δ_C 58.80 (59.40, *N*–*C*H–CH_3, 1-methin of the chain, 65/35 ratio of each intensity), 17.95 (18.01, 5-Me of the chain, about 50/50 ratio), the methin carbon at the 5-position (δ_C 32.7, d) not being split.

The ratios of the diastereomers for all the compounds described next were almost the same as those described for **5b**, **5c**, and **5d**.

N-(1,5,9-Trimethyldeca-4(Z),8-dienyl)-8-aza-4α,10-dimethyl-trans-decal-3-one (**6b**). HREIMS *m/z* (M^+): 359.3196; calcd. for $C_{24}H_{41}NO$: 359.3188.

N-(1,5,9-Trimethyldeca-4(E),8-dienyl)-8-aza-4α,10-dimethyl-trans-decal-3-one (**6c**). HREIMS *m/z* (M^+): 359.3177; calcd. for $C_{24}H_{41}NO$: 359.3188.

N-(1,5,9-Trimethyldecyl)-4α,10-dimethyl-8-aza-trans-decal-3-one (**6d**). HREIMS *m/z* (M^+): 363.3523; calcd. for $C_{24}H_{45}NO$: 363.3501.

N-(1,5,9-Trimethyldeca-4(Z),8-dienyl)-8-aza-4,4,10-trimethyl-trans-decal-3β-ol (**7b**). HREIMS *m/z* (M^+): 375.3524; calcd. for $C_{25}H_{45}NO$: 375.3501.

N-(1,5,9-Trimethyldeca-4(E),8-diethyl)-8-aza-4,4,10-trimethyl-trans-decal-3β-ol (**7c**). HREIMS *m/z* (M^+): 375.3520; calcd. for $C_{25}H_{45}NO$: 375.3501.

N-(1,5,9-Trimethyldecyl)-4,4,10-trimethyl-8-aza-trans-decal-3β-ol (**7d**). HREIMS *m/z* (M^+): 379.3816; calcd. for $C_{25}H_{49}NO$: 379.3814. *Anal.* Found: C, 79.46; H, 12.80; N, 3.36%. Calcd.: C, 79.09; H, 13.01; N, 3.69%.

N-(1,5,9-Trimethyldeca-4(Z),8-dienyl)-8-aza-4,4,10-trimethyl-trans-decal-3-one (**8b**). HREIMS *m/z* (M^+): 373.3379; calcd. for $C_{25}H_{43}NO$: 373.3345.

N-(1,5,9-Trimethyldeca-4(E),8-dienyl)-8-aza-4,4,10-trimethyl-trans-decal-3-one (**8c**). HREIMS *m/z* (M^+): 373.3377; calcd. for $C_{25}H_{43}NO$: 373.3345.

N-(1,5,9-Trimethyldecyl)-4,4,10-trimethyl-8-aza-trans-decal-3-one (**8d**). HREIMS *m/z* (M^+): 377.3660; calcd. for $C_{25}H_{47}NO$: 377.3658.

General procedure for syntheses of the 8-azadecalins coupled with fatty acids. Acyl chlorides were prepared with $SOCl_2$ from the fatty acids having C6, C9, C12, C15, or C18. A mixture of the fatty acids (0.22 mmol) and $SOCl_2$ (0.33 mmol) was refluxed for 3 h, before the excess $SOCl_2$ was removed by distillation. The acid chloride was added dropwise to a stirred solution of each acetylated decalin derivative in benzene (*ca.* 10 ml) containing triethylamine (22 mg). The reaction was allowed to continue for 5 h, and evaporation and column chromatography (SiO_2) with hexane–EtOAc = 10 : 1 gave the corresponding pure amides. The yield of each amide compounds in the **5**–**8** series was usually 60–80%. For the **5** and **7** series, the products were subjected to a deacetylation reaction, which was done by refluxing for 3 h with 1 N KOH in EtOH. The reaction mixture was concentrated *in vacuo* to a small quantity, water was added, and the solution was extracted with EtOAc. Chromatography on SiO_2 gave a pure amide, the deacetylation yield usually being 85–90%.

A pair of ^1H- and ^{13}C-signals for each proton and carbon of the B ring of the azadecalin, which was caused by the *cis/trans* isomerism of the amide group, was observed for all the amide compounds in a $CDCl_3$ solution, as described later. This means planarity for the B ring of the azadecalin, as has already been pointed out for compound **5g** in ref. 29. The ratio of *cis/trans* isomers for all the amide compounds was determined to be 1/1 by integrating ^1H-NMR data.

N-Pentadecanoyl-8-aza-4α,10-dimethyl-trans-decal-3β-ol (**5h**). NMR δ_H: 4.75 [3.89, (7eq.; 7 means the 7-position of decalin), ddd, $J=13$, 2.2, 2.2 Hz], 4.30 (3.40, 9 eq., dd, $J=12.8$, 2.0 Hz), 3.11 (not a pair, 3ax., ddd, $J=11$, 11, 5 Hz), 2.87 (2.36, 7ax., ddd, $J=13$, 13, 2.4 Hz), 2.56 (2.04, 9ax., d, $J=12.8$ Hz), around 2.2 (2 position of fatty acid, *t*-like, $J\cong 8$ Hz), 1.83 (2eq., m), 1.5–1.65 (CH_2 of fatty acid, br), 1.22 (CH_2 of fatty acid, s), 0.93 (4eq., CH_3, d, $J=6.5$ Hz), 0.81 (10ax., CH_3, s), 0.83 (CH_3 of fatty acid), 0.60 (5ax., ddd, $J=12$, 11, 3.6 Hz), other signals of the decalin being masked in the signals which appeared in the range 0.8–1.7 ppm; δ_C: 172.1 (s) [171.9 (s), *C*O], 77.18 (d) [77.10 (d), C3 of the decalin], 58.6 (t) [54.2 (t), C9], 48.2 (d) [48.1 (d), C5], 46.7 (t) [42.5 (t), C7], 38.8 (d) [38.6 (d), C4], 35.7 (t) [35.6 (t), C1], 34.8 (s) [34.4 (s), C10], 33.54 (t) [(33.15 (t), fatty acid C2], 31.9 (t, fatty acid), 30.5 (t) [30.4 (t), C2], 29.6 (t, fatty acid) 29.5 (t, fatty acid), 29.4 (t, fatty acid), 29.3 (t, fatty acid), 25.5 (t) [25.3 (t), fatty acid C3], 25.1 (t) [23.9 (t), C6], 22.6 (t, fatty acid), 16.1 (q) [16.0 (q), C10-CH_3], 14.6 (q) [14.5 (q), C4-CH_3], 14.1 (q) [C15-CH_3 of fatty acid]. HREIMS *m/z* (M^+): 407.3791; calcd. for $C_{26}H_{49}NO_2$: 407.3763. *Anal.* Found: C, 76.78; H, 12.31; N, 3.05%. Calcd.: C, 76.6; H, 12.11; N, 3.44%.

N-Dodecanoyl-8-aza-4α,10-dimethyl-trans-decal-3β-ol (**5g**). NMR δ_H values were almost the same as those of **5h**; δ_C 172.3 (s) [172.1 (s), *C*O], 77.18 (d) [77.10 (d), C3 of decalin], 58.6 (t), [54.3 (t), C9], 48.2 (d) [48.1 (d), C5], 46.7 (t) [42.5 (t), C7], 38.8 (d) [38.6 (d), C4], 35.7 (t) [35.6 (t), C1], 34.8 (s) [34.4 (s), C10], 33.6 (t) [(33.2 (t), fatty acid C2], 30.5 (t) [30.4 (t), C2], 31.9 (t, fatty acid), 29.6 (t, fatty acid) 29.5 (t, fatty acid), 29.4 (t, fatty acid), 29.3 (t, fatty acid), 29.2 (t, fatty acid), 29.1 (t, fatty acid), 25.5 (t) [25.3 (t), fatty acid C3], 25.1 (t) [23.9 (t), C-6], 22.6 (t, fatty acid), 16.1 (q) [16.0 (q), C10-CH_3], 14.6 (q) [14.5 (q), C4-CH_3], 14.1 (q) [C15-CH_3 of fatty acid]. HREIMS *m/z* (M^+): 365.3298; calcd. for $C_{23}H_{43}NO_2$: 365.3294.

N-Hexanoyl-8-aza-4α,10-dimethyl-trans-decal-3β-ol (**5e**). HREIMS *m/z* (M^+): 281.2352; calcd. for $C_{17}H_{31}NO_2$: 281.2355.

N-Nonanoyl-8-aza-4α,10-dimethyl-trans-decal-3β-ol (**5f**). HREIMS *m/z* (M^+): 323.2833; calcd. for $C_{20}H_{37}NO_2$: 323.2824. *Anal.* Found: C, 74.50; H, 11.20; N, 4.74%. Calcd.: C, 74.25; H, 11.53; N, 4.33%.

N-Nonanoyl-8-aza-4,4,10-trimethyl-trans-decal-3β-ol (**7e**). HREIMS *m/z* (M^+): 337.2962; calcd. for $C_{21}H_{39}NO_2$: 337.2981.

N-Dodecanoyl-8-aza-4,4,10-trimethyl-trans-decal-3β-ol (**7f**). HREIMS *m/z* (M^+): 379.3459; calcd. for $C_{24}H_{45}NO_2$: 379.3450. *Anal.* Found: C, 76.33; H, 11.60; N, 3.34%. Calcd.: C, 75.93; H, 11.95; N, 3.69%.

N-Pentadecanoyl-8-aza-4,4,10-trimethyl-trans-decal-3β-ol (**7g**). HREIMS *m/z* (M^+): 421.3950; calcd. for $C_{27}H_{51}NO_2$: 421.3920.

N-Dodecanoyl-8-aza-4α,10-dimethyl-trans-decal-3-one (**6g**). NMR: δ_H 4.76 (3.92, 7eq., ddd, $J=13.5$, 2.2, 2.2 Hz), 4.45 (3.52, 9eq., dd, $J=10.1$, 2.1 Hz), 2.90 (2.40, 7ax., dd, $J=12.1$, 2.4 Hz). 2.28 (not a pair, t, 2 position of fatty acid, t, $J=7.6$ Hz), 2.63 (2.14, d, $J=12.1$ Hz), 1.45–1.63 (CH_2 of fatty acid, br), 1.25 (CH_2 of fatty acid, s), 1.05 (10ax., CH_3, d, $J=11$ Hz), 0.95 (4ax., CH_3, d, $J=6.5$ Hz), 0.82 (CH_3 of fatty acid, t, $J=6.6$ Hz), 0.62

(5ax., ddd, $J=12$, 11, 3.6 Hz), other signals of the decalin being masked in the signals which appeared in the range of 1.2–1.8 ppm; δ_C: 211.5 (s) (211.2, C3 of decalin), 178.5 (s), 172.3 (s) [172.2 (s), CONH], 57.9 (t) [53.6 (t), C9], 50.1 (d) [50.0 (d), C5], 46.0 (t) [41.9 (t), C7], 44.8 (d) [44.6 (d), C4], 37.6 (t) [37.5 (t), C1], 37.3 (t) [37.2 (t), C2], 34.9 (t) [34.5 (t), C10], 34.0 (t) (fatty acid) 33.5 (t) [33.1 (t), C2 of fatty acid), 31.0 (t) (fatty acid), 29.3 (t) (signals of CH_2 of fatty acids), 26.3 (t) (fatty acid), 25.5 (t) (fatty acid), 25.2 (t) [25.1 (t), C6], 24.7 (t) (fatty acid), 22.6 (t) (fatty acid), 15.5 (q) [15.1 (q), C10-CH_3], 14.0 (q) (fatty acid, CH_3), 10.9 (q) [10.8 (q), C4-CH_3]. HREIMS m/z (M^+): 363.3141; calcd. for $C_{23}H_{41}NO_2$: 363.3137. *Anal.* Found: C, 75.72; H, 11.52; N, 3.96%. Calcd.: C, 75.98; H, 11.37; N, 3.85%.

N-Hexanoyl-8-aza-4α,10-dimethyl-trans-decal-3-one (**6e**). HREIMS m/z (M^+): 279.2194; calcd. for $C_{17}H_{29}NO_2$: 279.2198.

N-Nonanoyl-8-aza-4α,10-dimethyl-trans-decal-3-one (**6f**). HREIMS m/z (M^+): 321.2671; calcd. for $C_{20}H_{35}NO_2$: 321.2667. *Anal.* Found: C, 74.96; H, 11.01; N, 4.20%. Calcd.: C, 74.72; H, 10.97; N, 4.36%.

N-Pentadecanoyl-8-aza-4α,10-dimethyl-trans-decal-3-one (**6h**). HREIMS m/z (M^+): 405.3610; calcd. for $C_{26}H_{47}NO_2$: 405.3606.

N-Octadecanoyl-8-aza-4α,10-dimethyl-trans-decal-3-one (**6i**). HREIMS m/z (M^+): 447.4073; calcd. for $C_{29}H_{53}NO_2$: 447.4076.

N-Dodecanoyl-8-aza-4,4,10-trimethyl-trans-decal-3-one (**8e**). HREIMS m/z (M^+): 377.3290; calcd. for $C_{24}H_{43}NO_2$: 377.3294.

Preparation of the amines from the corresponding amides. A solution of a corresponding amide (**5e–i** and **7e–g**) in ether was refluxed for 3 h with an excess of $LiAlH_4$ (10 equiv.). To the suspension was added EtOAc saturated with water, and the solution was stirred overnight to quench the activity of $LiAlH_4$. After filtration, the organic layer was subjected to SiO_2 column chromatography to give a pure product (hexane–EtOAc saturated with NH_3). The yield of each of amine was 85–90%. These amines did not have the pair of ^{13}C-signals found in corresponding amides **5e–i** and **7e–g**.

N-Pentadecyl-8-aza-4α,10-dimethyl-trans-decal-3β-ol (**5m**). NMR: δ_H 3.08 (3ax., ddd, $J=11$, 10, 5 Hz), 2.92 (7eq., ddd, $J=11$, 2, 2 Hz), 2.49 (9eq., dd, $J=10$, 1.7 Hz), 2.05–2.4 (N–CH_2 of side chain, AB spin system), 1.76 (7ax., ddd, $J=11$, 11, 3.5 Hz), 1.4–1.7 (CH_2 of side chain, m), 1.34 (9ax., d, $J=10$ Hz), 1.22 (s, CH_2 of side chain), 0.98 (10ax., CH_3, s), 0.91 (4eq., CH_3, d, $J=6.5$ Hz), 0.85 (CH_3 of side chain, t, $J=6.2$ Hz), 0.64 (5ax., ddd, $J=12$, 11, 3.6 Hz), other decalin signals being masked in the other signals of 0.8–1.7 ppm; δ_C 76.9 (s) (C3 of decalin), 67.6 (t) (C9), 58.8 (t) (either C7 or fatty acid), 55.7 (t) (either C7 or fatty acid), 48.0 (d) (C5), 38.9 (d) (C4), 36.7 (t) (C1), 34.0 (s) (C10), 31.9 (t, fatty acid), 30.6 (t) (C2), 29.6 (t, fatty acid), 29.3 (t, fatty acid), 27.5 (t, fatty acid), 27.0 (t, fatty acid), 25.1 (t, C6), 22.7 (t, fatty acid), 18.0 (q, C10 CH_3), 14.7 (q, C4 CH_3), 14.1 (q, fatty acid CH_3). HREIMS m/z (M^+): 393.3956; calcd. for $C_{26}H_{51}NO_2$: 393.3971. *Anal.* Found: C, 79.25; H, 13.04; N, 3.31%. Calcd.: C, 79.32; H, 13.06; N, 3.56%.

N-Hexyl-8-aza-4α,10-dimethyl-trans-decal-3β-ol (**5j**). HREIMS m/z (M^+): 267.2568; calcd. for $C_{17}H_{33}NO$: 267.2562.

N-Nonanyl-8-aza-4α,10-dimethyl-trans-decal-3β-ol (**5k**). HREIMS m/z (M^+): 309.3051; calcd. for $C_{20}H_{39}NO$: 309.3032.

N-Dodecyl-8-aza-4α,10-dimethyl-trans-decal-3β-ol (**5l**). HREIMS m/z (M^+): 351.3486; calcd. for $C_{23}H_{45}NO$: 351.3501. *Anal.* Found: C, 78.30; H, 12.71; N, 3.75%. Calcd.: C, 78.57; H, 12.90; N, 3.98%.

N-Octadecyl-8-aza-4α,10-dimethyl-trans-decal-3β-ol (**5n**). HREIMS m/z (M^+): 435.4444; calcd. for $C_{29}H_{57}NO$: 435.4440.

N-Nonanyl-8-aza-4,4,10-trimethyl-trans-decal-3β-ol (**7h**). HREIMS m/z (M^+): 323.3189; calcd. for $C_{21}H_{41}NO$: 323.3188.

N-Dodecyl-8-aza-4,4,10-trimethyl-trans-decal-3β-ol (**7i**). HREIMS m/z (M^+): 365.3661; calcd. for $C_{24}H_{47}NO$: 365.3658. *Anal.* Found: C, 78.96; H, 12.84; N, 3.86%. Calcd.: C, 78.84; H, 12.96; N, 3.84; N, 3.83%.

N-Pentadecyl-8-aza-4,4,10-trimethyl-trans-decal-3β-ol (**7j**). HREIMS m/z (M^+): 407.4118; calcd. for $C_{27}H_{53}NO$: 407.4127. *Anal.* Found: C, 79.53; H, 13.20; N, 3.26%. Calcd: C, 79.54; H, 13.1; N, 3.44%.

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