Human Pituitary Growth Hormone. XXXI. The Synthesis of Two Protected Peptide Fragments Occurring in the Region of Residues 53–67

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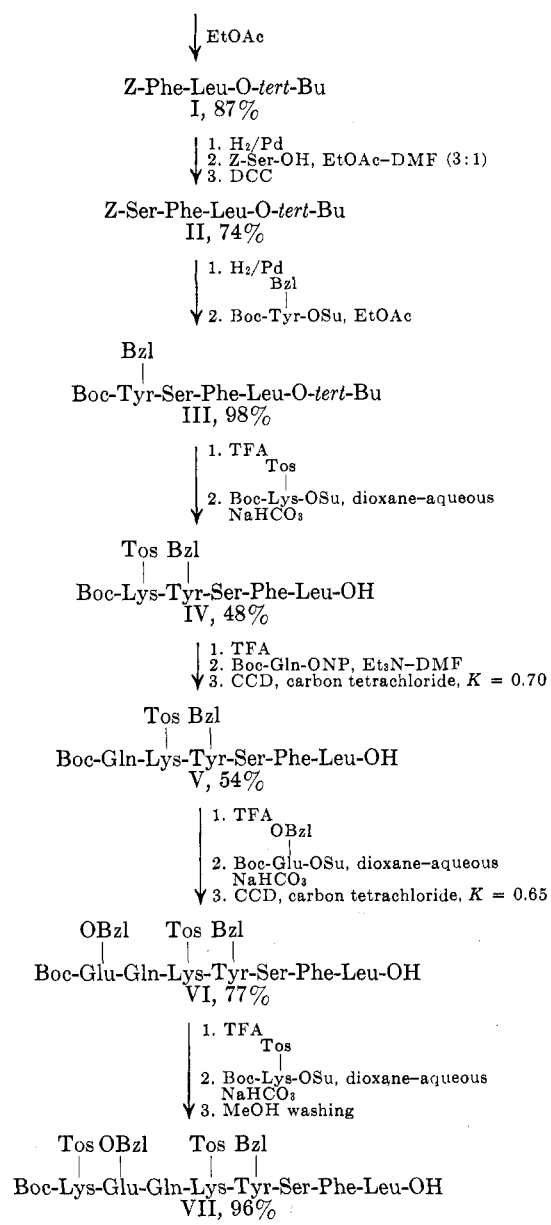
Two protected peptide fragments occurring in the region of residues 53–67 in the HGH molecule have been synthesized. These peptides are Boc-Lys(Tos)-Glu(OBzl)-Gln-Lys(Tos)-Tyr(Bzl)-Ser-Phe-Leu-OH and Boc-Gln-Asp(OBzl)-Pro-Glu(OBzl)-Thr-Ser-Leu-OH.

In a previous report,[1] we described the synthesis of three protected peptides occurring in the region of residues 53–67 in the amino acid sequence[2,3] of the HGH molecule.[4] This paper presents an alternative synthesis of this pentadecapeptide as two fragments, an octa- and a heptapeptide, in highly purified form and in excellent yields. They are, respectively, N^{α}-*tert*-butyloxycarbonyl-N^{ϵ}-tosyllysyl-γ-benzylglutamylglutaminyl-N^{ϵ}-tosyllysyl-*O*-benzyltyrosylserylphenylalanylleucine[5] (VII) and *tert*-butyloxycarbonylglutaminyl-β-benzylaspartylprolyl-γ-benzylglutamylthreonylserylleucine (XIII).

Scheme I illustrates the synthesis steps for the octapeptide corresponding to residues 53–60 of HGH and Scheme II for the heptapeptide corresponding to residues 61–67. Leucine *tert*-butyl ester[6] was coupled with benzyloxycarbonylphenylalanine *N*-hydroxysuccinimide ester[7] in ethyl acetate to obtain crystalline benzyloxycarbonylphenylalanylleucine *tert*-butyl ester (I). After catalytic hydrogenolysis, the free base dipeptide ester was coupled with benzyloxycarbonylserine using dicyclohexylcarbodiimide[8] (DCC) to obtain the crystalline protected benzyloxycarbonylserylphenylalanylleucine *tert*-butyl ester (II). The tripeptide II was catalytically hydrogenolyzed to remove the benzyloxycarbonyl group and then coupled with *tert*-butyloxycarbonyl-*O*-benzyltyrosine *N*-hydroxysuccinimide ester[9] to yield the crystalline protected tetrapeptide *tert*-butyloxycarbonyl-*O*-benzyltyrosylserylphenylalanylleucine *tert*-butyl ester (III) in high yield. Peptide III was then treated with trifluoroacetic acid to remove both N- and C-protecting groups. Reaction of the deblocked tetrapeptide with the *p*-nitrophenyl ester of N^{α}-*tert*-butyloxycarbonyl-N^{ϵ}-tosyllysine gave a low yield (10%) of pentapeptide N^{α}-*tert*-butyloxycarbonyl-N^{ϵ}-tosyllysyl-*O*-benzyltyrosylserylphenylalanylleucine (IV). However, when the *N*-hydroxysuccinimide ester of N^{α}-*tert*-butyloxycarbonyl-N^{ϵ}-tosyllysine was used for coupling, the yield of pentapeptide IV was up to 48%. Peptide IV was treated with trifluoroacetic acid to remove the *tert*-butyloxycarbonyl group and then reacted with *tert*-butyloxycarbonylglutamine *p*-nitrophenyl ester[10] to yield the hexapeptide *tert*-butyloxycarbonylglutaminyl-N^{ϵ}-tosyllysyl-*O*-benzyltyrosylserylphenylalanylleucine (V). In attempts to syn-

SCHEME I
SYNTHESIS OF THE PROTECTED OCTAPEPTIDE VII



(1) K. Kovacs, Y. Kovacs-Petres, and C. H. Li, *Int. J. Protein Res.*, **3**, 93 (1971).

(2) C. H. Li, W.-K. Liu, and J. S. Dixon, *J. Amer. Chem. Soc.*, **88**, 2050 (1966).

(3) C. H. Li, J. S. Dixon, and W.-K. Liu, *Arch. Biochem. Biophys.*, **133**, 70 (1969).

(4) Abbreviations: HGH, human pituitary growth hormone; DCC, dicyclohexylcarbodiimide; Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; *tert*-Bu, *tert*-butyl; OSu, *N*-hydroxysuccinimide ester; Bzl, benzyl; Tos, tosyl; ONP, *p*-nitrophenyl ester; tlc, thin layer chromatography; CCD, countercurrent distribution; TFA, trifluoroacetic acid; DMF, dimethylformamide; NMM, *N*-methylmorpholine.

(5) All the amino acids occurring in the peptides mentioned in this paper are of the L configuration.

(6) R. Roeske, *J. Org. Chem.*, **28**, 1251 (1963).

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(9) M. Low and L. Kisfaludy, *Acta Chim. Acad. Sci. Hung.*, **44**, 61 (1965).

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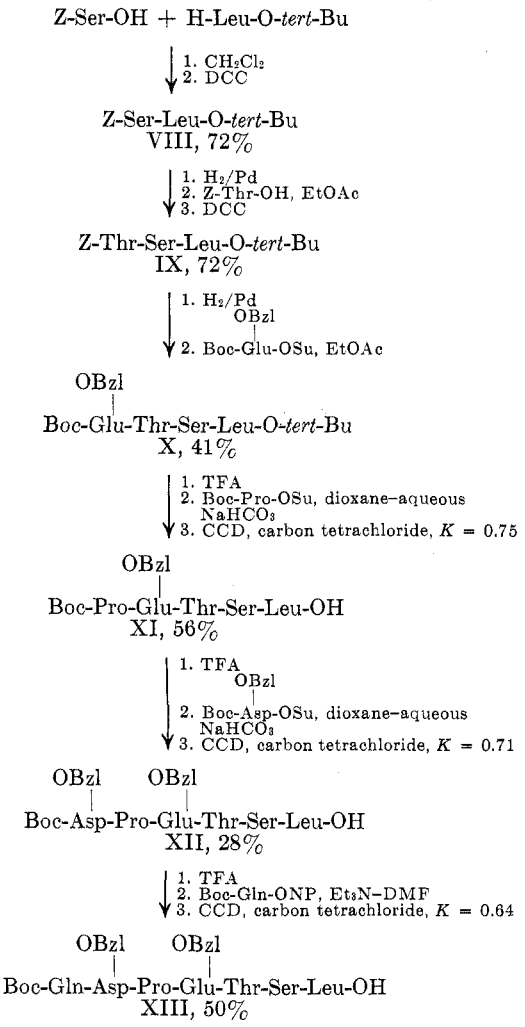
SCHEME II

SYNTHESIS OF THE PROTECTED HEPTAPEPTIDE XIII



thesize V *via* the *N*-hydroxysuccinimide ester of *tert*-butyloxycarbonylglutamine[11] very low yields (5%) were obtained, caused perhaps by the rapid intramolecular cyclization of the activated ester.[12] The protected hexapeptide V was treated with trifluoroacetic acid and then coupled with *tert*-butyloxycarbonyl-γ-benzylglutamic acid *N*-hydroxysuccinimide ester[13] to produce the protected heptapeptide *tert*-butyloxycarbonyl-γ-benzylglutamylglutaminyl-N^{ϵ}-tosyllysyl-*O*-benzyltyrosylserylphenylalanylleucine (VI). Peptide VI was deblocked with trifluoroacetic acid and coupled with N^{α}-*tert*-butyloxycarbonyl-N^{ϵ}-tosyllysine *N*-hydroxysuccinimide ester to yield N^{ϵ}-*tert*-butyloxycarbonyl-N^{ϵ}-tosyllysyl-γ-benzylglutamylglutaminyl-N^{ϵ}-tosyllysyl-*O*-benzyltyrosylserylphenylalanylleucine (VII).

The heptapeptide corresponding to residues 61–67 of HGH was synthesized by first coupling benzyloxycarbonylserine to leucine *tert*-butyl ester using DCC to form benzyloxycarbonylserylleucine *tert*-butyl ester (VIII). The protected dipeptide was subjected to catalytic hydrogenolysis to remove the benzyloxycarbonyl group and then coupled with benzyloxycarbonylthreonine using DCC to yield benzyloxycarbonylthreonylserylleucine *tert*-butyl ester (IX). Peptide IX was freed from the protecting group by catalytic hydrogenolysis and the resulting free tripeptide ester was allowed to react with the *N*-hydroxysuccinimide ester of *tert*-butyloxycarbonyl-γ-benzylglutamic acid to yield *tert*-butyloxycarbonyl-γ-benzylglutamylthreonylserylleucine *tert*-butyl ester (X). Tetrapeptide X was treated with trifluoroacetic acid to remove the *tert*-butyloxycarbonyl and *tert*-butyl ester groups, and subsequent condensation with *tert*-butyloxycarbonylproline *N*-hydroxysuccinimide ester[7] yielded *tert*-butyloxycarbonylprolyl-γ-benzylglutamylthreonylserylleucine (XI). This pentapeptide derivative was deprotected by treatment with trifluoroacetic acid and then coupled with *tert*-butyloxycarbonyl-β-benzylaspartic acid *N*-hydroxysuccinimide ester[14] to form *tert*-butyloxycarbonyl-β-benzylaspartylprolyl-γ-benzylglutamylthreonylserylleucine (XII). Finally, the protected hexapeptide was deprotected with trifluoroacetic acid and coupled with *tert*-butyloxycarbonylglutamine *p*-nitrophenyl ester to yield *tert*-butyloxycarbonylglutaminyl-β-benzylaspartylprolyl-γ-benzylglutamylthreonylserylleucine (XIII). Peptide XIII was purified by countercurrent distribution and found to be homogeneous in thin layer chromatography in several systems.

Experimental Section

Melting points were determined on a Fisher-Johns block and are uncorrected. All samples for microanalysis[15] were dried in an Abderhalden pistol over phosphorus anhydride at 77° for 16 hr at 0.3 mm. Thin layer chromatography on silica gel was carried out with the following solvent systems: methanol–chloroform (1:1), acetone–chloroform (1:1), and 1-butanol–acetic acid–water (4:1:1). Peptide spots were located by the ninhydrin reagent and by the chlorine method.[16] Countercurrent distribution was performed on a 100-tube, all-glass apparatus with a capacity of 50 cc for each phase. Analysis of the material was by weight determination after removal of an aliquot and subsequent evaporation and drying. The solvent systems used were toluene, toluene–chloroform–methanol–water (5:5:8:2), and carbon tetrachloride, carbon tetrachloride–chloroform–methanol–water (1:3:3:1). Catalytic hydrogenolysis was performed in the presence of palladium (6–10 mmol of peptide per 50 ml of methanol), freshly prepared[17] from palladium chloride, by means of a Vibro-Mixer[18] in the apparatus described by Meienhofer.[19] Acidolytic cleavage of *tert*-butyloxycarbonyl groups was done as follows. The peptide is dissolved in trifluoroacetic acid (1 mmol per 15 ml of acid). After being stirred at room temperature for 20 min, the trifluoroacetic acid was removed *in vacuo* and the residue was triturated repeatedly with absolute methanol followed by evaporation *in vacuo* to remove traces of trifluoroacetic acid.

Schemes I and II describe in detail the conditions used in the synthesis of the octa- and heptapeptides. Concentrations for the coupling reactions were as follows. The DCC couplings, 12–20 mmol per 100 ml of solvent, and equimolar quantities of each reactant were used. The *p*-nitrophenyl and *N*-hydroxysuccinimde ester reactions, 6–10 mmol per 100 ml of solvent with a 10% excess of the active ester, were used. Purification of the various peptides followed procedures described previously,[20] *i.e.*, the filtration, evaporation, and washings normally utilized in peptide synthesis. The physical properties and analytical description of each peptide fragment are presented in Tables I and II.

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(13) W. O. Danho and C. H. Li, *Int J. Protein Res.*, **3**, 99 (1971).
(14) See ref 13.
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TABLE I

PHYSICAL DATA OF SYNTHETIC FRAGMENTS FOR THE SYNTHESIS OF PROTECTED OCTAPEPTIDE VII[a]

Compd	State (solvent, mp, °C)	R_f[b] A	B	C	$[\alpha]^{25}D$, deg
Z-Phe-Leu-O-*tert*-Bu (I)	Crystalline (petroleum ether, 94~95)	0.85	0.75	0.64	−27.0 (*c* 1, methanol)
Z-Ser-Phe-Leu-O-*tert*-Bu (II)	Crystalline (EtOAc, 160–161)	0.86	0.77	0.56	−39.2 (*c* 1, methanol)
Bzl Boc-Tyr-Ser-Phe-Leu-O-*tert*-Bu (III)	Crystalline (EtOAc–petroleum ether, 99~100)	0.87	0.71	0.52	−24.6 (*c* 1, methanol)
Tos Bzl Boc-Lys-Tyr-Ser-Phe-Leu-OH (IV)	Crystalline (EtOAc, 148–150)	0.86	0.62	0	−33.9 (*c* 1, methanol)
Tos Bzl Boc-Gln-Lys-Tyr-Ser-Phe-Leu-OH (V)	Amorphous (196–198)	0.82	0.60	0	−23.0 (*c* 1, methanol)
OBzl Tos Bzl Boc-Glu-Gln-Lys-Tyr-Ser-Phe-Leu-OH (VI)	Amorphous (195–197)	0.80	0.58	0	−26.2 (*c* 1, methanol)
Tos OBzl Tos Bzl Boc-Lys-Glu-Gln-Lys-Tyr-Ser-Phe-Leu-OH (VII)[c]	Amorphous (205–209)	0.75	0.55	0	−30.1 (*c* 1, DMF)

[a] Elementary analyses (C, H, N) for all compounds in the table were within ±0.3% of calculated values. [b] Solvent systems: A, 1-butanol–acetic acid–water (4:1:1); B, chloroform–methanol (1:1); C, chloroform–acetone (1:1). [c] Amino acid analysis of VII (theoretical, found) by acid hydrolysis: Lys (2.00, 1.81); Ser (1.00, 0.91); Glu (2.00, 2.06); Leu (1.00, 1.04); Tyr (1.00, 0.48); Phe (1.00, 1.02).

TABLE II

PHYSICAL DATA OF SYNTHETIC FRAGEMNTS FOR THE SYNTHESIS OF PROTECTED HEPTAPEPTIDE XIII[a]

Compd	State (solvent, mp, °C)	R_f[b] A	B	C	$[\alpha]^{25}D$, deg
Z-Ser-Leu-O-*tert*-Bu (VIII)	Crystalline (EtOAc–ether, 91–93)	0.87	0.74	0.63	−41.5 (*c* 1, methanol)
Z-Thr-Ser-Leu-O-*tert*-Bu (IX)	Crystalline (chloroform–ether, 120–121)	0.85	0.75	0.50	−36.5 (*c* 1, methanol)
OBzl Boc-Glu-Thr-Ser-Leu-O-*tert*-Bu (X)	Crystalline EtOAc, 117–119)	0.86	0.70	0.52	−32.4 (*c* 1, methanol)
OBzl Boc-Pro-Glu-Thr-Ser-Leu-OH (XI)	Amorphous (95–100)	0.71	0.69	0.49	−43.2 (*c* 1, methanol)
OBzl OBzl Boc-Asp-Pro-Glu-Thr-Ser-Leu-OH (XII)	Amorphous (100–102)	0.80	0.65	0.20	−35.1 (*c* 1, methanol)
OBzl OBzl Boc-Gln-Asp-Pro-Glu-Thr-Ser-Leu-OH (XIII)[c]	Amorphous (115–118)	0.75	0.57	0.10	−56.2 (*c* 1, methanol)

[a] Elementary analyses (C, H, N) for all compounds in the table were within ±0.3% of calculated values except as follows: VIII, N within 0.35%; X, C within 0.4%; XII, C within 0.6%; XIII, N within 0.45%. [b] Solvent systems: A, 1-butanol–acetic acid–water (4:1:1); B, chloroform–methanol (1:1); C, chloroform–acetone (1:1). [c] Amino acid analysis of XIII (theoretical, found) by acid hydrolysis: Asp (1.00, 0.98); Thr (1.00, 0.96); Ser (1.00, 0.93); Glu (2.00, 2.05); Pro (1.00, 1.06); Leu (1.00, 1.02).

N^{α}-*tert*-Butyloxycarbonyl-N^{ϵ}-tosyllysine *N*-Hydroxysuccinimide Ester.—N^{α}-*tert*-Butyloxycarbonyl-N^{ϵ}-tosyllysine cyclohexylamine salt (4.99 g, 10 mmol) and *N*-hydroxysuccinimide (1.15 g, 10 mmol) were dissolved in 45 ml of dioxane–ethyl acetate (3 l., v/v) with stirring and cooled to 3~5°. Dicyclohexylcarbodiimide (2.06 g, 10 mmol) was added and the mixture was stirred overnight at 3~5°. Filtration to remove dicyclohexylurea was followed by evaporation to dryness. The residue was dissolved in 2-propanol (50 ml) and kept in the refrigerator for 1 week. It crystallized out slowly. Filtration yielded the

activated ester (3.13 g, yield 62.8%), mp 114–117°, $[\alpha]^{26}D$ −19.1° (c 1, methanol).

Anal. Calcd for $C_{22}H_{31}N_3O_8S$ (497.6): C, 53.10; H, 6.28; N, 8.45. Found: C, 53.74; H, 6.48; N, 8.43.

Registry No.—I, 29842-94-2; II, 29842-95-3; III, 29842-96-4; IV, 29842-97-5; V, 29842-98-6; VI, 29842-99-7; VII, 29843-00-3; VIII, 28252-48-4; IX, 29843-02-5; X, 29843-03-6; XI, 29843-04-7; XII, 29843-05-8; XIII, 29843-06-9; N^{α}-*tert*-butyloxycarbonyl-N^{ϵ}-tosyllysine *N*-hydroxysuccinimide ester, 29843-07-0.

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The Structure of Paradisiol, a New Sesquiterpene Alcohol from Grapefruit Oil

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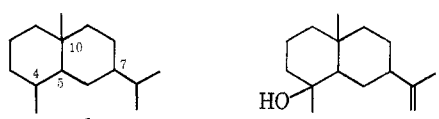
Paradisiol, a new sesquiterpene alcohol isolated from grapefruit peel oil, was shown to be 5β*H*,7β,10α-selin-11-en-4α-ol (**8**).

Previous investigations of grapefruit oil (*Citrus paradisi* Swingle) led to the detection of a bicyclic sesquiterpene ketone, nootkatone, which is considered to be the principal flavoring constituent.[1,2] Although it is the major oxygenated sesquiterpene (0.3%) in the oil, it occurs with a number of other compounds of which no detailed information is yet available.[3] Since further flavor contributors may be expected in this group, we started a composition study of a grapefruit oil fraction which was rich in nootkatone and contained a multitude of other components. In this paper we report the isolation and chemical structure of a new sesquiterpenic alcohol, named paradisiol (**8**).

Paradisiol (**8**), mp 85–86°, $C_{15}H_{26}O$, shows tertiary hydroxyl bands at 3612 and 3480 cm^{-1} and terminal methylene bands at 3090, 1635, and 890 cm^{-1}. The proton resonance spectrum shows two tertiary methyl groups at δ 0.93 (s, 3 H) and 1.04 (s, 3 H), one vinylic methyl at δ 1.74 (s, 3 H), and one methylene group at δ 4.84 (s, 2 H). On catalytic hydrogenation with palladium/C in acetic acid or with platinum/C in ethanol, 1 mol of hydrogen was consumed.

Paradisiol was readily dehydrated with phosphorus oxychloride in pyridine to give a mixture of two isomeric olefins (**9**, **10**) which could be separated by gas chromatography. Both the major (**9**) and the minor (**10**) dehydration products show bands in the infrared for terminal methylene (3080, 1642, 888 and 3090, 1640, 890 cm^{-1}, respectively). The nmr spectrum of **9** contains signals for a tertiary methyl group at δ 0.73 (s, 3 H) and a vinylic methyl group at δ 1.70 (s, 3 H). In addition, two terminal methylene groups at δ 4.37 (s, 1 H), 4.63 (s, 1 H), 4.77 (s, 1 H), and 4.85 (s, 1 H) are shown. The nmr spectrum of **10** shows a tertiary methyl group at δ 0.83 (s, 3 H) and two vinylic methyl groups at δ 1.56 (s, 3 H) and 1.71 (s, 3 H). It further shows a total of three olefinic protons at δ 4.80 (s, 2 H) and 5.22 (s, 1 H), the latter one being attached to a trisubstituted double bond.

Paradisiol is thus a bicyclic tertiary alcohol, bearing an isopropenyl group. Hydrogenation of either **9** or **10**, Pd/C in acetic acid, was shown by gas chromatography to yield an identical tetrahydro derivative. This hydrocarbon was shown to be identical with selinane (eudesmane) (**1**), prepared from β-selinene. The gross structure of paradisiol can therefore be written as selin-



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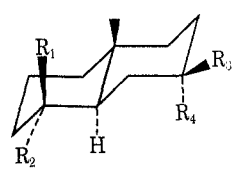
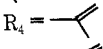


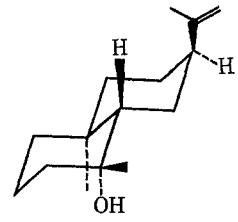
3, $R_1 = CH_3$; $R_2 = OH$; R_3 =  ; $R_4 = H$

4, $R_1 = OH$; $R_2 = CH_3$; R_3 =  ; $R_4 = H$

5, $R_1 = CH_3$; $R_2 = OH$; $R_3 = H$; R_4 = 

6, $R_1 = OH$; $R_2 = CH_3$; $R_3 = H$; R_4 = 

7, $R_1 = CH_3$; $R_2 = H$; R_3 =  ; $R_4 = H$



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11-en-4-ol (**2**). In analogy with β- and α-selinene, "β" and "α" are used to designate the position of the double bond in **9** and **10**, respectively.

All naturally occurring compounds of the selinane family hitherto reported are *trans*-decalin derivatives. Assuming paradisiol to be trans also (*vide infra*), the discussion on the relative configuration can be confined

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