Stereoselective Reactions. XVIII. 1) Synthesis and Cytotoxicity of the Demethyl Derivatives of Steganes

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Demethyl derivatives of steganes and deoxypodorhizon, 3, 4, 6, 7, 9, 10, 12, 13, 18, 23, were prepared by the selective demethylation of the methoxy group of steganes and deoxypodorhizon, 2, 5, 8, 11, 22. The cytotoxicity of these derivatives was evaluated against KB cell and was found not to exceed that of the parent steganes. 4-Demethyldeoxypodorhizon (18) was found to show more potent cytotoxicity than deoxypodorhizon (22).

Keywords stegane; cytotoxicity, selectivity; synthesis; demethylation; lignan

We have been involved in these years in the asymmetric total synthesis of steganin lignans (for example steganacin (1)) and have found that isopicrosteagane (11), one of the four stegane stereoisomers, 2, 5, 8, 11, shows a promising cytotoxicity against KB cell.2-3) The mechanism of action was found to involve inhibition of microtubule assembly, as has been reported in the case of podophyllotoxin (14).4-5) On the other hand, demethylpodophyllotoxin (15) has been reported to show higher cytotoxicity than its parent compound and to inhibit deoxyribonucleic acid (DNA) replication.6) Its derivative has been used clinically in cancer chemotherapy. We report herein the synthesis and cytotoxicity of 10- and 11-demethyl derivatives of steganes (3, 4, 6, 7, 9, 10, 12, 13) and 3- and 4-demethyideoxypodorhizon (18, 23).6)

Oxidative Coupling of 19 To synthesize demethyl derivatives of steganes our study began with the synthesis and nonphenolic oxidative coupling of 19. Conjugate addition reaction of the lithiated piperonal diphenylthioacetal (16) with butenolide and trapping of the resulting enolate with 4-benzoxo-3,5-dimethoxybenzyl bromide provided 17.6) Reductive desulfurization of 17 and benzylation of 18 afforded 19. Oxidative coupling of 19 with the use of VOF3 in methylene chloride–trifluoroacetic acid provided a mixture of 20 and 21 in a ratio of 5:1 in a low yield of 7%. Debenzylation by hydrogenolysis and subsequent

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methylation provided isostegane (8) and stegane (2), respectively. It is quite interesting to note that in contrast to the oxidation of 22 stereoselectively providing 8 in an excellent yield, oxidation of 19 gave rise to a mixture of stereoisomers in a quite low yield.

**Direct Demethylation of 22 and Steganes**

Since the conversion of 19 to 20 was not efficient, we turned our attention to the synthesis of demethylsteganes by direct demethylation of steganes. Treatment of 22 with chlorotrimethylsilane (TMSCl) and sodium iodide in acetonitrile \(^1\) was found to produce 18 in 42% yield along with 23 in 3% yield. The structure of the major product was confirmed by direct comparison with 18 obtained by reduction of 17. Application of these conditions to demethylation of steganes provided the desired demethylsteganes. The results of the reaction are summarized in Table I.

In the demethylation reaction of steganes, two isomers were, however, obtained in comparable yields. The position of demethylation was assigned based on the direct comparison with 9 and 3 obtained from 20 and 21, and Hückel molecular orbital calculations.

As shown in Fig. 1, the linear combination of atomic orbital (LCAO) coefficients of highest occupied molecular orbital (HOMO) of A indicate that oxygen of the 4-methoxy group in 22 is more reactive than that at the 3- or 5-position. This is in good agreement with the result of demethylation of 22. On the other hand, B, which reflects steganes (2, 5, 8, 11), \(^1\) indicates that the methoxy groups of the two positions have similar reactivity, affording two demethylated products in equal amounts, and thus supporting the structures of the isomers obtained from steganes. Since demethylation would take place by the initial attack of oxygen of the methoxy group on the silicon atom (C), the LCAO coefficients well reflect the differences in reactivity.

**Isomerization and Correlation of Demethylsteganes**

The structures of 10- and 11-demethylsteganes were determined by applying a combination of selective thermal atropisomerization and base-induced epimerization at the \(x\)-position to the lactone carbonyl. \(^7\) Since the structures of 10-demethylisostegane (9) and 10-demethylstegane (3) were confirmed by conversion of 20 and 21, respectively, other demethylisomers were correlated with 9 and 3. Thus thermal atropisomerization of 9 at 215 °C for 2 h produced a 1:1 mixture of 9 and 3. Upon treatment of 9 with 10% aqueous NaOH in benzene for 15 min at room temperature and subsequent acidification with 10% aqueous HCl for lactonization, epimerization at the \(x\)-position to the lactone carbonyl group took place to afford a mixture of 3 and 10-demethylisocrostegane (6). Thermal atropisomerization of 6 produced a mixture of 6 and 10-demethylisocrostegane (12).

That the structures of 10-demethylsteganes were determined as above supports the assigned structures of 11-demethylsteganes, because the 11-demethyl series were derived from the corresponding steganes.

**Cytotoxicity of Demethylsteganes and Demethyldeoxygenopodorhizon**

The cytotoxicity of these compounds was evaluated against KB cell. The results are summarized in Table II. Demethylsteganes show weaker cytotoxicity than their parent steganes (2, 5, 8, 11), and the isopicrostegane derivatives (12, 13) were the most active, as in the case of the parent stegane (11). It is quite interesting that 4-demethyldeoxygenopodorhizon (18) shows more potent activity than deoxyopodorhizon (22).

Further studies aimed at the development of potent antitumor compounds are in progress in our laboratory. \(^1\) \(^3\)

**Experimental**

Melting points were measured using a Büchi 510 melting point apparatus and are not corrected. Infrared (IR) spectra were taken with a Jasco infrared spectrometer, model DS-402G. Proton nuclear magnetic resonance (\(^1\)H-NMR) spectra were taken in CDCl3 unless otherwise noted with a JNM-PS100 spectrometer, or with a JEOL FX100 spectrometer at 100 MHz, or with a Hitachi R-24 spectrometer at 60 MHz. Chemical shift values are expressed in ppm relative to internal tetramethylsilane. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Mass spectra (MS) were taken with a JEOL JMS-SX 100, 102 mass spectrometer, or with a JEOL DX-300 mass spectrometer.

**4-Benzylxoy-3,5-dimethoxybenzyl Bromide**

Phosphorus tribromide (1.9 ml) was added dropwise to a solution of 4-benzylxoy-3,5-dimethoxybenzyl alcohol \(^1\) (13.3 g, 48.4 mmol) in ether (1 l) under ice-water bath cooling. The mixture was stirred at room temperature for 12 h. The whole was washed with sat. NaHCO3 and brine. Concentration afforded colorless needles which were recrystallized from benzene–hexane to afford the corresponding bromide (14.3 g) as colorless needles of mp 58—59°C. NMR \(\delta:\) 3.78 (6H, s, CH3O), 4.40 (2H, s, CH2Br), 4.97 (2H, s, CH3Ph), 6.52 (2H, s, Ar-H). MS m/z: 338 (M+2), 336 (M+). Anal. Caled for C24H19BrO6: C, 56.99; H, 5.08. Found: C, 56.84; H, 5.01.

\((\pm)-3,3′-(1,1′-Diphenylthiopiperinyl)-2-(4-benzylxoy-3,5-dimethoxy)benzyl]-4-butanolide\)](17) A hexane solution of BuLi (11.7 ml,
16.5 mmol) was added to a cooled (−78 °C) solution of 16 (6.34 g, 18.0 mmol) in tetrahydrofuran (THF, 75 ml) and the mixture was stirred for 30 min. A solution of y-butenolide (1.26 g, 15.0 mmol) in THF (30 ml) was added to the above solution and the whole was stirred for 1 h. After the addition of hexamethyldisilazane (HMDS) (6.45 g, 36.0 mmol) and 4-benzyloxy-3,5-dimethoxybenzyl bromide (5.06 g, 15.0 mmol) in THF (30 ml), the mixture was stirred for 22 h at room temperature. Purification of the reaction mixture using a flash column with silica gel (230−400 mesh) and elution with a mixture of hexane diethyl ether (8:1) afforded 17 (3.95 g, 93%) as a colorless gum. IR (KBr): 3420, 1764, 1605 cm−1. MS m/z: 384 (M+), C21H20O4. Yield: 384.1206.

**General Procedure for Demethylated Exemplified by the Synthesis of (+)-trans-4-Demethylideoxypodorhizone (18) and (+)-trans-3-Demethylideoxypodorhizone (23)**

To a solution of 22 (200 mg, 0.50 mmol) in acetone/tritile (1:1 ml) was added sodium iodide (150 mg, 1 mmol) and TMSICl (109 mg, 1 mmol). The mixture was stirred at room temperature for 5 h and then water (10 ml) was added. The mixture was extracted with ethyl acetate. The extract was washed with water containing 10% NaOH and brine, and then was dried over MgSO4. Concentration afforded a yellow oil (220 mg). Purification by column chromatography (eluent: hexane/ethyl acetate 5:1) afforded 22 (72 mg, 36% recovery). IR (KBr): 3420, 1764, 1605 cm−1. MS m/z: 384 (M+), C21H20O4. Yield: 384.1206.

**(-)-10-Demethylstegane (3) and (+)-11-Demethylstegane (4)** Prepared from stegane (2) according to the general procedure. Yields are given in Table I.

4. Colorless prism (methyl) of mp 215−216 °C. NMR (CD3OD) δ: 1.46 and 3.86 (each 3H, CH2-CH3). 4.19 and 4.66 (1H, CH, 0.61 (2H, CH, OCH2). 6.74 and 6.75 (1H, CH). IR (KBr): 3560, 1764, 1605 cm−1. MS m/z: 384 (M+), C21H20O4. Yield: 384.1208.

**(-)-10-Demethylpicrostegane (6) and (+)-11-Demethylpicrostegane (7)** Prepared from picrostegane (5) according to the general procedure. Yields are given at 55 °C in Table I.

6. Colorless prism (methyl) of mp 211−212 °C. NMR (CD3OD) δ: 0.8−3.06 (7H, m, CH), 3.20 and 3.34 (each 3H, s, CH3). 3.47−4.85 (1H, CH, 0.61 (2H, CH, OCH2). 6.74 and 6.75 (1H, CH). IR (KBr): 3560, 1764, 1605 cm−1. MS m/z: 384 (M+), C21H20O4. Yield: 384.1212.

**(+)-10-Demethylbicyclosisostegane (9) and (+)-11-Demethylbicyclosisostegane (10)** Prepared from isostegane (8) according to the general procedure. Yields are given in Table I.


10. Colorless prism (methyl) of mp 278 °C. NMR (CD3OD) δ: 3.40 and 3.90 (each 3H, CH2-CH3). 3.86−4.15 (1H, CH, 0.61 (2H, CH, OCH2). 6.74 and 6.75 (1H, CH). IR (KBr): 3340, 1768, 1688 cm−1. MS m/z: 384 (M+), C21H20O4. Yield: 384.1217.

**(+)-10-Demethylisopicrostegane (12) and (+)-11-Demethylisopicrostegane (13)** Prepared from isopicrostegane (11) according to the general procedure. Yields are given in Table I.

12. Colorless prism (methyl) of mp 205−208 °C. NMR (CD3OD) δ: 3.42 and 3.46 (each 3H, s, CH3). 4.29 (1H, CH, 0.61 (2H, CH, OCH2). 6.51 and 6.52 (1H, CH, Ar-H). IR (KBr): 3340, 1777, 1606 cm−1. MS m/z: 384 (M+), C21H20O4. Yield: 384.1208.


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References and Notes

NII-Electronic Library Service
4) Unpublished results.


6) Cytotoxic activity of steganes is highly dependent on the absolute configuration around the pivotal bond and the compounds described in this paper are the active forms (unpublished results). All compounds illustrated in this paper are racemic.


10) The biphenyl ring system of steganes can be treated as alkyl-aromatic bonds, because the dihedral angle between the two aromatics is over 60° and no significant conjugative contribution is possible.

