Analysis of Methylation Product——The product (20 mg) was dissolved in the mixture of 3 ml of methanol and 1 ml of water containing oxalic acid (40 mg). The solution was heated in a sealed tube at 75° for 20 hr, then neutralized with $CaCO_3$ and filtered. The filtrate was evaporated to dryness, then dissolved in 0.5% methanolic HCl (2 ml) and left at room temperature for 16 hr. The solution was treated with Amberlite IR4B (OH⁻) to remove HCl, then evaporated to dryness. This methanolysate was used for the identification of methyl ethers of D-fructose. For the detection of methyl ether of D-glucose, further treatment with 4% methanolic HCl was carried out in a sealed tube at 75° for 16 hr, followed by removal of HCl. Chloroform solution of the methanolysate was applied to a gas chromatograph. Following two conditions were used; A, a column (0.3 cm × 2 m long stainless steel) packed with 15% Poly-butane 1,4-diol succinate on Chromosorb W (80 to 100 mesh) at 175° with a flow of 20 ml per min of N_2; B, a column (0.3 cm × 2 m long stainless steel) packed with 5% Neopentylglycol succinate on Chromosorb G (60 to 80 mesh) at 150° with a flow of 20 ml per min of N_2. Table I shows relative retention times of the products obtained by methanolysis to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside in the two conditions.

TABLE I. Relative Retention Times of Methylation Products

	Condition A (15% BDS)	Condition B (5% NPGS)
Methyl 2,3,4,6-tetra-O-methyl D-glucoside	1.00, 1.36	1.00, 1.43
Methyl 1,3,4,6-tetra-O-methyl D-fructoside	1.05, 1.24	1.07, 1.28
Methyl 3,4,6-tri-O-methyl D-fructoside	2.44, 3.55	2.47, 3.67

Studies on the Syntheses of Heterocyclic Compounds. DX.[1]
A Novel Rearrangement of proerythrinadienol with Methyl Fluorosulfonate

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We have previously reported that phenol oxidation and irradiation of the phenolic 1-benzylisoquinolines gave proerythrinadienones, (I)[3a] and (II),[3b] respectively. This type of compound (I) is proved to be the precursors in biosynthesis of the erythrina[4] (III) and the aporphine alkaloids (IV).[5] Battersby also suggested that the dienone like I would be the precursor to hasubanan type alkaloids (VII).[6] Furthermore, dienone-phenol rearrangement of dienone (II) was investigated under various kinds of conditions, but failed.[3b] Therefore, rearrangement of this dienone (II) to thalicsimidine-type aporphine (V) was examined

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5) A.R. Battersby, J.L. MuHugh, J. Staunton, and M. Todd, *Chem. Commum.*, 1971, 985.
6) A.R. Battersby, "Oxidative Coupling of Phenols," ed. A.R. Battersby and W.I. Taylor, Marsel Dekker, New York, 1967, p. 119.

by treatment with methyl fluorosulfonate, by the result of which a negative result was also obtained. On the other hand, the dienol (VIII) was treated with methyl fluorosulfonate to give an aporphine (VI) in low yield in addition to a dienone (IX), which was reduced with lithium aluminum hydride in tetrahydrofuran (THF) to afford an enone (XI) but not the dienol (XII). The oxidized product (X) of the latter dienol (XII) would be a key intermediate for protostephanine.[7]

The structural assignment of the aporphine (VI) was achieved by spectroscopical method as follows; the ultraviolet (UV) spectrum showed the typical aporphine absorption and nuclear magnetic resonance (NMR) spectrum also supported the presence of this system by an aromatic proton resonanced at 2.10 τ, in low field, in addition to the other two aromatic protons.

Thus an interesting rearrangement of proerythrinadienol to an aporphine with methyl fluorosulfonate has been achieved.
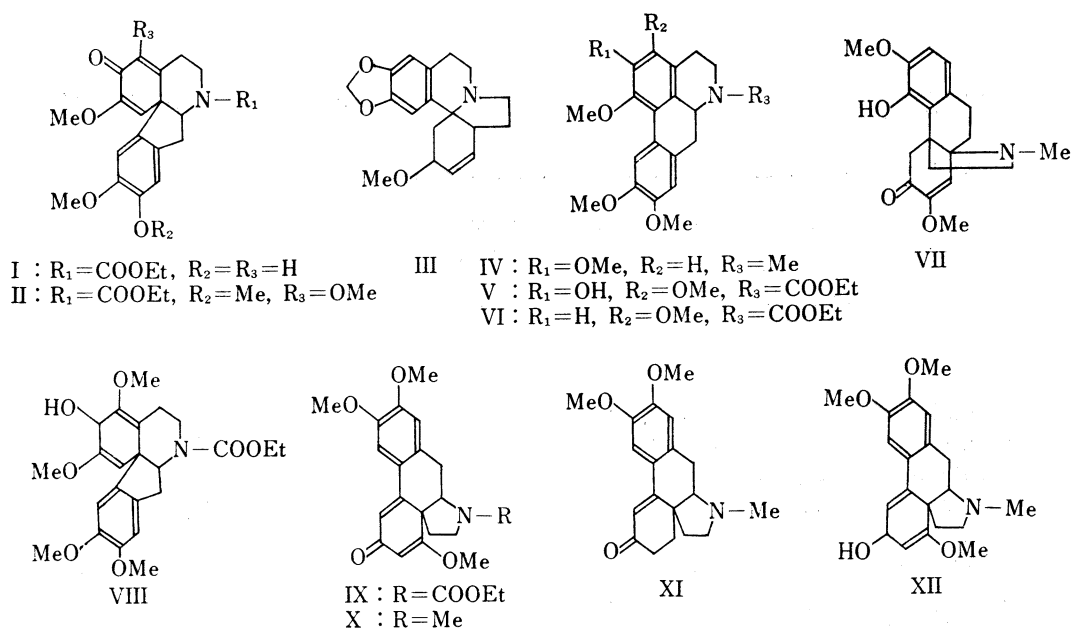

I : $R_1=COOEt$, $R_2=R_3=H$
II : $R_1=COOEt$, $R_2=Me$, $R_3=OMe$

III

IV : $R_1=OMe$, $R_2=H$, $R_3=Me$
V : $R_1=OH$, $R_2=OMe$, $R_3=COOEt$
VI : $R_1=H$, $R_2=OMe$, $R_3=COOEt$

VII

VIII

IX : $R=COOEt$
X : $R=Me$

XI

XII

Chart 1

Experimental[8]

Rearrangement of the Dienol (VIII) with Methyl Fluorosulfonate——A mixture of 0.35 g of dienol (VIII)[3b] and 3 g of methyl fluorosulfonate was stirred for 2 hr at 15—18°. After decomposition of the excess of methyl fluorosulfonate with sat. $NaHCO_3$ aq. solution, the resulting mixture was extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and evaporated to leave an orange syrup, which was chromatographed on 10 g of silica gel using $CHCl_3$ as an eluant to give 3 mg of the aporphine (VI) as colorless granules (from benzene–hexane), mp 142—143°.

Anal. Calcd. for $C_{23}H_{27}O_6N$: C, 66.81; H, 6.58; N, 3.39. Found: C, 66.56; H, 6.51; N, 3.33. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1672. UV λ_{max}^{MeOH} nm (log ε): 278 (4.15), 307 (4.16), 315 (4.13). NMR (CDCl$_3$) τ: 2.10 (1H, s, C_{11}-H), 3.28 (1H, s, C_8-H), 3.57 (1H, s, C_2-H), 5.81 (2H, q, $J=7$ Hz, CH$_2$CH$_3$), 6.10 (3H, s, OCH$_3$), 6.14 (6H, s, 2 × OCH$_3$), 6.18 (3H, s, OCH$_3$), 8.72 (3H, t, $J=7$ Hz, CH$_2$CH$_3$).

7) A.R. Battersby, A.K. Bhatnagar, P. Hackett, C.W. Thornber, and J. Staunton, *Chem. Commun.*, **1968**, 1214.
8) Infrared (IR) and ultraviolet (UV) spectra were taken with type EPI-2 and EPS-3 Hitachi recording spectrophotometers, respectively. Mass spectra were measured with a Hitachi RMU-7 mass spectrometer, and NMR spectra were taken with a Hitachi H·60 with tetramethylsilane as an internal standard.

The second $CHCl_3$ eluant gave 120 mg of the dienone (IX) as colorless prisms, mp 167—168° (from benzene–hexane), the spectroscopic data of which were identical with the authentic sample.[3b]

Reduction of the Dienone (IX) with Lithium Aluminum Hydride——A mixture of 0.1 g of dienone (IX) and 50 mg of $LiAlH_4$ in 30 ml of dry THF was refluxed for 1.5 hr. After evaporation of the solvent, the residue was carefully made basic with 5% NH_4OH and then extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and evaporated to dryness. Chromatography of the product on 3 g of silica gel using $CHCl_3$–MeOH (99 : 1) as the eluant gave 50 mg of enone (XI) as colorless prisms (from benzene–hexane), mp 141—142°. *Anal.* Calcd. for $C_{19}H_{23}O_3N$: C, 72.82; H, 7.40. Found: C, 72.79; H, 7.35. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1645. Mass Spectrum *m/e*: 313 (M$^+$), 256. NMR (CDCl$_3$) τ: 3.12, 3.30, 3.97 (3H, each s, two aromatic and one olefinic protons), 6.10 (3H, s, OC$\underline{H}_3$), 6.14 (3H, s, OC$\underline{H}_3$), 7.62 (3H, s, NC$\underline{H}_3$).

A New Semi-alkaline Proteinase produced by *Streptomyces cinereoruber*[1]

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Semi-alkaline proteinases having optimum pH between 8 and 9 of *Streptomyces* origin have been reported as products of *Streptomyces erythreus*,[3] *Streptomyces fradiae*[4] and *Streptomyces madurae*.[5] The proteolytic activity of all of the above semi-alkaline proteinases can be inactivated by addition of diisopropylfluorophosphate. We have recently isolated a new semi-alkaline proteinase from the cultured-broth of *Streptomyces* H 55- SY 7 which was isolated from a soil sample collected at Kochi prefecture and identified as *Streptomyces cinereoruber*.[6] The proteolytic activity of the new semi-alkaline proteinase is not inactivated by addition of diisopropylfluorophosphate, but inactivated by addition of ethylenediaminetetraacetate.

Production, isolation, purification and enzymatic characteristics of the proteinase are presented in this paper.

The semi-alkaline proteinase was produced by cultivation of *Streptomyces* H 55-SY 7 in shaking flasks containing a medium composed of starch, glucose, soy bean meal, yeast extract and various inorganic salts on a reciprocal shaker. The proteinase was precipitated from the broth filtrate by saturation with ammonium sulfate and the crude enzyme mixture was reprecipitated by addition of cold acetone after dialysis against water. The enzyme was purified by gradient column chromatography on DEAE-cellulose after gel filtration

1) This is Part VIII of "Anti-inflammatory Proteinases obtained from Streptomyces" by S. Nakamura. Part VII: S. Nakamura, H. Yamashita, T. Shimoda, H. Fukuda, M. Hamada, M. Matsuzaki, and H. Umezawa, *Chem. Pharm. Bull.* (Tokyo), **21**, 584 (1973).
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