to the periodate-benzidine test.[7] Their NMR spectra were characteristic for the structures IVb and VI (Table I). In addition, 2′,3′-O-isopropylidene derivatives of (S)-2,5′, and (N)-2,5′-anhydronucleosides (IVb and VI) were also prepared from 2′,3′-O-isopropylidene derivatives of IIIb and V in a similar way.

So far as is known, the work reported here is the first specifically designed to synthesize 2,5′-anhydro pyrimidine nucleosides directly from pyrimidine nucleosides. The studies of cleavage reaction of (S)-2,5′-and (N)-2,5′-anhydronucleosides are being undertaken.

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Syntheses and Properties of Formyl Sarcosine[1]-LH-RH and N-Methyl-L-pGlu[1]-LH-RH[1)]

In the course of our investigations on the structure-activity relationship of luteinizing hormone-releasing hormone (LH-RH), pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, acylated Gly[1]-LH-RH such as formyl Gly[1]-LH-RH was revealed to retain some biological activity in spite of the lack of the N-terminal pyrrolidone ring structure.[2)] Time course study based on the serum level of luteinizing hormone (LH) after the administration of formyl Gly[1]-LH-RH in rats suggested shorter life time of this compound than that of LH-RH in animal body (Fig. 1). In an attempt to suppress the rapid inactivation, we synthesized formyl Sar[1]-LH-RH, in which methyl group was introduced to the nitrogen atom of formyl glycine part, and found a marked increase in LH-releasing activity (Table I). A prolonged high LH-level in serum was also observed distinctly (Fig. 1). This result prompted us to synthesize N-methyl-L-pGlu[1]-LH-RH, and its LH-releasing activity remained at a half level of the natural LH-RH.

These analogs, formyl Sar[1]-LH-RH and N-methyl-L-pGlu[1]-LH-RH, were synthesized as follows. The protected nonapeptide amide, Z-His-Trp-Ser(Bu^t)-Tyr(Bu^t)-Gly-Leu-Arg(Tos)-Pro-Gly-NH_2 (I), synthesized according to the same procedure described in our previous communication,[2)] was decarbobenzoxylated by hydrogenolysis and coupled with formyl sarcosine *p*-nitrophenyl ester to give the protected decapeptide amide (II) [mp 165—167°, $[\alpha]_D^{20}$ −33.3° (c=0.47 in MeOH), *Anal.* Calcd. for $C_{69}H_{97}O_{15}N_{17}S \cdot 1.5H_2O$: C, 56.62; H, 6.89; N, 16.27. Found: C, 56.90; H, 6.97; N, 15.58]. Deprotection of II with anhydrous hydrogen fluoride,[3)] followed by purification with column partition chromatography on Sephadex G-25

1) The amino acid residues (except glycine and sarcosine) mentioned in this communication are of the L-configuration. The abbreviations used to denote amino acids, peptides and peptide derivatives are those recomended by IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, **5**, 2485 (1966); **6**, 362 (1967).
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Table I. LH-Releasing Activity of the Synthesized Analogs

	% LH-releasing activity	95% confidence limits
LH-RH (synthetic)	assumed 100%	
Formyl Gly^1-LH-RH	1.5%	0.3— 7.8
Formyl Sar^1-LH-RH	52.6%	66.2—41.8
N-methyl-L-pGlu^1-LH-RH	48.4%	73.3—32.0

(solvent system: *n*-BuOH: 0.1M $AcONH_4$=1: 1) produced chromatographically homogeneous formyl Sar^1-LH-RH [$[\alpha]_D^{20}$ −69.4° (*c*=0.32 in 0.1N AcOH), Rf^1 0.41, Rf^2 0.51, Rf^3 0.66.[4] Amino acid ratios (acid hydrolysate): Sar 1.09, His 0.93, Trp 0.71, Ser 0.82, Tyr 0.89, Gly 1.85, Leu 1.00, Arg 1.00, Pro 1.04. *Anal.* Calcd. for $C_{54}H_{75}O_{13}N_{17}\cdot 2AcOH\cdot 2H_2O$: C, 52.52; H, 6.61; N, 17.95. Found: C, 52.69; H, 7.27; N, 18.14].

N-Methyl-L-pGlu^1-LH-RH was prepared by the coupling of N-methyl-L-pyroglutamic acid 1-succinimidyl ester with the deprotected nonapeptide amide (III) which was derived from II by treatment with anhydrous hydrogen fluoride. The resulting decapeptide amide (IV) was purified by column partition chromatography on Sephadex G-25 (solvent system: *n*-BuOH: 0.1M $AcONH_4$: CCl_4=10: 10: 1) to give the pure material [$[\alpha]_D^{20}$ −54.9° (*c*=0.27 in 0.1N AcOH), Rf^1 0.39, Rf^2 0.51, Rf^3 0.63. Amino acid ratios (acid hydrolysate): His 0.92, Trp 0.78, Tyr 0.97, Ser, 0.81, Gly, 2.00, Leu, 1.00, Arg, 0.98, Pro, 1.01. *Anal.* Calcd. for $C_{56}H_{77}O_{13}N_{17}\cdot 2AcOH\cdot 7H_2O$: C, 49.96; H, 6.92; N, 16.51. Found: C, 49.83; H, 6.88; N, 16.21]. N-Methyl-L-pyroglutamic acid[5] [mp 150—151°, $[\alpha]_D^{20}$ −33.6° (*c*=1.1 in H_2O), *Anal.* Calcd. for $C_6H_9O_3N$: C, 50.34; H, 6.34; N, 9.79. Found: C, 50.08; H, 6.27; N, 9.74] used herein, was obtained by resolution[6] of N-methyl-DL-pyroglutamic acid[7] with quinidine.

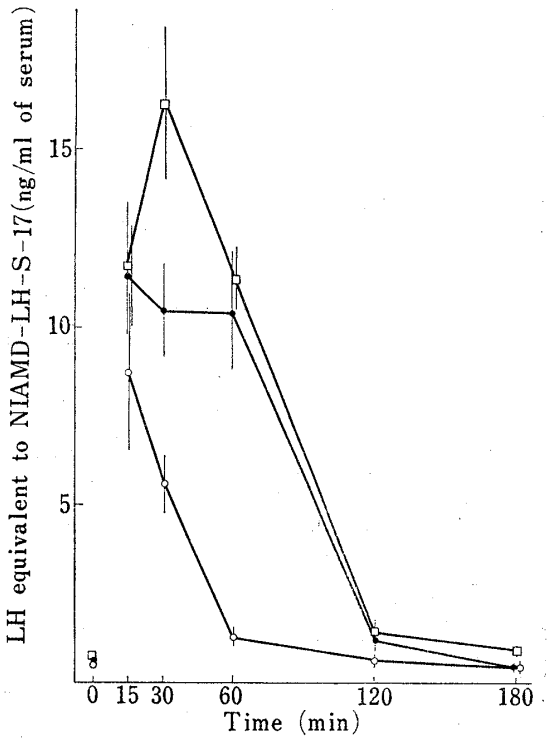

Fig. 1. Serum LH Concentrations (mean ± SE) after *s.c.* Injection of LH-RH and Its Analogs

s.c. injection of LH-RH and its analogs
—●—: LH-RH (0.5 μg per rat)
—○—: formyl Gly^1-LH-RH (50 μg per rat)
—□—: formyl Sar^1-LH-RH (1 μg per rat)

The LH-releasing activity of the synthesized analogs was estimated at two dose levels by the stimulation of release of LH in ovariectomized rats pretreated with estrogen and progesterone.[8] The duration of the LH-releasing activity was evaluated by a single *s.c.* injection in male immature rats (25—27 days

4) *Rf* values of thin-layer chromatography refer to the following solvent systems: Rf^1, *n*-BuOH–AcOH–H_2O (60: 15:25); Rf^2, *n*-BuOH–AcOH–H_2O–pyridine (30: 6: 24: 20); Rf^3, $CHCl_3$–MeOH–32% AcOH (60: 45: 20).

5) The absolute configuration of this N-methyl-pyroglutamic acid was determined to be of the L-configuration by hydrolyzing to known N-methyl-L-glutamic acid (J.C. Watkins, *J. Med. Pharm. Chem.*, **5**, 1187 (1962)).

6) N-Methyl-L-pyroglutamic acid-quinidine salt: mp 201—202°, $[\alpha]_D^{20}$ +158° (*c*=1.0 in EtOH), *Anal.* Calcd. for $C_{26}H_{33}O_5N_3$: C, 66.79; H, 7.11; N, 8.99. Found: C, 66.80; H, 6.80; N, 9.06.

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old).[9] The serum LH concentrations were measured by radioimmunoassay according to Niswender, *et al.*[10]

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The Absolute Configurations of Pterosins, 1-Indanone Derivatives from Bracken, *Pteridium aquilinum* var. *latiusculum*[1]

In the previous communications[2–4] the isolation and the structural elucidations of seventeen pterosins, sesquiterpenoids having 1-indanone nucleus, from methanol extract of air-dried young leaves of bracken were reported. This communication concerns with the absolute configurations of these compounds.[1]

Pterosin B[2] (**1**) was proved to be identical with the aglycone of pteroside B[5] (**2**) and the absolute configuration at C-2 was proposed to be *R* on the basis of the circular dichroism (CD) curve by Hikino.[5] Since the direct application of the method to the indanone system is assumed to have some limitation,[6] the confirmation by an unequivocal method was carried out as follows.

The ozonolysis of pterosin B (**1**) afforded methylsuccinic acid (**3**), mp 109—111°, which showed $[\alpha]_D$ +7.8° (H_2O) and a positive Cotton effect (peak, 218 nm), indicating its *R*-configuration.[7] This result showed the *R*-configuration of the 2-position of pterosin B (**1**).

The *trans*-configuration of the methyl at C-2 and the hydroxyl at C-3 in pterosin C[3] (**4**) was shown in the previous communications.[3,4] The Clemmensen reduction of pterosin C (**4**) gave a product (**5**), mp 68°, $[\alpha]_D$ +3.0°, $[\alpha]_{350}$ +10.5° (MeOH), which was proved to be identical with the Clemmensen reduction product[2] (**6**) of pterosin B, mp 67—68°, $[\alpha]_D$ −2.0°,

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