which was synthesized by the method of Wolfrom and Anno[11]. The reaction mixture (2 ml) contained 50 mM potassium phosphate buffer (pH 7.5), 2.5 mM L-gulono-γ-lactone, 1 mM EDTA, and liver microsomes of about 3 mg of protein equivalents. The mixture was incubated for 30 min at 37 °C, and the reaction was stopped by the addition of 2 ml of 10% trichloroacetic acid. After the protein had been precipitated, L-ascorbic acid in the supernatant was determined by the method of Roe and Kuether[9]. Protein was determined by the method of Lowry et al.[12] with a slight modification.

Results and discussion. Table 1 shows the L-ascorbic acid concentrations in the rat organ tissues. Only trace amounts of L-ascorbic acid were found in the liver, kidney and adrenal glands of the 3-week-old rats with osteogenic disorder (homozygotes, *od*/*od*) compared with those of the littermates, of which the external appearance was normal (heterozygotes, +/*od* and normal +/+). The levels in the OD rats decreased still further at 4 weeks of age. Table 2 shows the activities of L-gulonolactone oxidase in the liver microsomal fractions of OD rats and the littermates of normal phenotype. The littermates had the enzyme activity, but rats with the disorder did not. Figure 2 shows the growth curves of rats with the disorder given tap water or water containing L-ascorbic acid (40 mg/100 ml) to drink from weaning. In the rats given tap water, hind limb disorders were observed after 3 weeks, body weight gain stopped after 4 weeks and death due to incomplete development occurred after 7 to 8 weeks. However, the rats given L-ascorbic acid grew up almost normally and were fertile (table 3).

In the homozygous OD rat, disorder of the hind limbs becomes clear from 15–16 days of age and mere traces of the long bones of the 4 limbs and deformed epiphyseal cartilages are observed from 4 weeks of age at autopsy. Hematomas around the femurs, humerus and scapulae are also observed[7]. These observations resemble the symptoms of infantile scurvy. Collagen is one of the main structural materials of bone, and L-ascorbic acid is essential for the biosynthesis of collagen[13]. Therefore, we determined the tissue levels of this vitamin. Only traces of L-ascorbic acid were found in rats with the osteogenic disorder compared with the levels in the littermates of normal phenotype and normal rats of other substrains. Usually rats can synthesize L-ascorbic acid[3-5] and a dietary supply is not necessary. Therefore, the commercial diet for rats contains lower amounts of vitamin C than that for guinea pigs. Our analysis showed that the vitamin C content in the diet, disinfected at 100 °C for 30 min, was about 20 mg/kg. Addition of L-ascorbic acid to the drinking water prevented development of the osteogenic disorder in the homozygotes and the rats grew up normally and were fertile. These observations clearly proved that the homozygous of OD rat, like primates and guinea pigs, can not synthesize L-ascorbic acid. The enzyme system catalyzing the conversion of L-gulono-γ-lactone into L-ascorbic acid is located entirely in the liver microsomal fraction in rats[14]. The littermates of normal phenotype had the enzyme activity but rats with the osteogenic disorder did not. This shows that the homozygotes of the OD rat, like primates and guinea pigs, lack L-gulonolactone oxidase (EC 1.1.3.8).

We conclude that homozygous OD rats do not have L-ascorbic acid-synthesizing ability because of a lack of L-gulonolactone oxidase (EC 1.1.3.8). These rats with a hereditary defect in L-ascorbic acid-synthesizing ability should be useful not only for nutritional and pharmacological studies on vitamin C, but also for genetic studies on the lack of L-gulonolactone oxidase.

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Specific inhibition of human leukocyte elastase by substituted alpha-pyrones[1]

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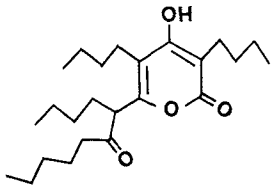
Summary. Several α-pyrones have been synthesized and investigated for their in vitro inhibitory activity using α-chymotrypsin (α-CT), porcine pancreatic elastase (PPE) and human leukocyte elastase (HLE). 4-Hydroxy-6-undecyl-2H-pyran-2-one **4**, 4-Hydroxy-6-[(1-butyl)heptyl]-2H-pyran-2-one **5** and 4-Methoxy-6-[(1-butyl)heptyl]-2H-pyran-2-one **6** were found to be specific inhibitors of HLE. These compounds constitute a promising new class of HLE inhibitors.

The chronic destruction of the elastic component of lung connective tissue by human leukocyte elastase (HLE) and cathepsin G is currently believed to result in the onset of chronic obstructive lung disease[2-6]. These proteases are primarily inhibited by the major serum proteases inhibitor α_1-proteinase inhibitor (α_1-PI), which is also a normal constituent of bronchioalveolar lavage fluid (BAL). However, α_1-PI is readily inactivated by oxidants such as those present in cigarette smoke or oxidative enzymes (i.e. myeloperoxidase) that are normally functioning in phagocytic cells during inflammatory states. In addition, some individuals are genetically deficient in α_1-PI with levels of the inhibitor which are 25% of normal.

Thus, individuals with a compromised inhibitor sceen are prime candidates for chromic obstructive lung disease[7].

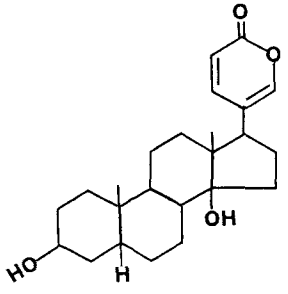
Our long-term goal has been the development of potent and biospecific inhibitors of HLE and their use in the treatment of pulmonary emphysema and related ailments[8-10]. We report here the results of a study aimed at a) developing readily accessible and biospecific inhibitors of HLE resembling elasnin (**I**) – a naturally-occurring inhibitor of HLE elaborated by the microorganism *Streptomyces noboritoensis*[11-13] and b) delineating the structural features in elasnin that are responsible for its inhibitory activity toward HLE.



I

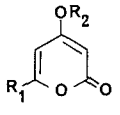
Materials and methods. Compounds **2–5** of the table were prepared by alkylating 6-Methyl-4-hydroxy-2H-pyran-2-one **1** (Aldrich Chemical Co.)[14]. Compound **6** was obtained by methylating compound **5**[14]. All compounds exhibited satisfactory spectral data and gave correct elementary analyses. Porcine pancreatic elastase (Worthington) and α-chymotrypsin (Sigma) were assayed as described previously[8-10]. Human leukocyte elastase was isolated as described by Powers[15] and was assayed according to Bieth using succinyl-tri-L-ala-p-nitroanilide[2]. The inhibition studies were carried out as follows: 7 µl of HLE (3.29×10^{-5} solution in Tris-HCl buffer, pH 8.0), 7 µl of spectrograde acetonitrile and 25 µl of Tris-HCl buffer were pipetted into a thermostatted cuvette maintained at 25 °C and allowed to equilibrate for 20 min. 1 ml of substrate, succinyl-tri-L-ala-p-nitroanilide, 2.22×10^{-3} M in 0.2 M Tris-HCl buffer, was added and the change in absorbance at 410 nm was recorded over a period of 2 min using a Gilford 2600 uv/visible spectrophotometer equipped with a Hewlett-Packard X-Y plotter. Repetition of the experiment in the presence of each inhibitor (a 200-fold excess of inhibitor was used) in spectrograde acetonitrile and assaying the enzyme activity after the addition of succinyl-tri-L-ala-p-nitroanilide indicated the extent of inhibition.

Results and discussion. The incubation of compounds **3** through **6** in the table with HLE resulted in significant inhibition of the enzyme. Compound **5**, the closest congener of elasnin (**I**), was found to be the most active. The C-4 methoxy-substituted compound **6** was not as effective in inhibiting the enzyme. This might be due to the fact that the C-4 hydroxyl group serves as locus of hydrogen bonding interactions thereby enhancing binding. The length of the alkyl chain at C-6 – which reflects the hydrophobicity of the molecule – is of paramount importance in this class of compounds. Alkyl chains that are either too short (compounds **1** and **2** (Table) or too bulky (compound (**II**)) yielded inactive compounds. The inhibitory activity of these compounds appears to reside primarily in the hydrophobic side chain at C-6. HLE prefers hydrophobic substrates and has been postulated[16] to have an elongated hydrophobic cavity far from the active site. It is conceivable that the hydrophobic side chain at C-6 binds to this cavity inducing a conformational change in the enzyme, in accord with the previously reported inhibitory activity of a series of unsaturated fatty acids[16]. The active compounds cited in the table had no effect on PPE and α-CT. The interesting biological activity of these compounds warrants further study and the results of our studies in this area will be reported soon.



II

Substituted alpha-pyrones



Compound	R_1	R_2	% Inhibition[a]
1	CH_3	H	NA[b]
2	$CH_3(CH_2)_2$	H	NA[b]
3	$CH_3(CH_2)_6$	H	36.9
4	$CH_3(CH_2)_{10}$	H	49.1
5	$CH_3(CH_2)_5-CH(CH_2)_3CH_3$	H	61.4
6	$CH_3(CH_2)_5-CH(CH_2)_3CH_3$	CH_3	27.6

[a] Under the conditions stated in the 'Materials and methods' section.
[b] NA = no activity.

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