ment of the hydrolysis of 2-diethylaminoethyl *p*-nitrobenzoate[7] and *p*-nitrophenyl acetate[8] by liposomes. They explained the former in terms of partition theory and the latter in terms of reaction with lecithin. The mechanism of the enhanced inactivation of elastase by REV remains to be clarified, but it is important to note that the enhancement depends upon the method of preparation of liposomes.

The comparative studies of SML and REV showed that REV provides greatly superior encapsulation and retards the inactivation of elastase in the neutral pH region. Therefore, as Szoka and Papahadjopoulos[3] suggested, the REV method could be very useful for the preparation of carriers to encapsulate not only elastase but also other macromolecules. However, as shown in Fig. 3 or Fig. 5, the REV preparation accelerated the inactivation of the enzyme in the lower pH region, which was not enountered in the case of the SML preparation. The finding that the physicochemical properties of the liposomes depend upon their method of preparation suggests that further and more precise studies on the methods of preparation are required.

Acknowledgement This work was supported in part by scientific research funds from the Ministry of Education, Science and Culture, Japan.

References and Notes

1) B.E. Ryman, R.F. Jewkes, K. Jeyasingh, M.H.N. Tattersall, and D.A. Tyrrell, *Ann. N.Y. Acad. Sci.*, **308**, 281 (1978); G. Gregoriadis, *ibid.*, **308**, 343 (1978); *etc.*
2) C.M. Colley and B.E. Ryman, *Biochim. Biophys. Acta*, **451**, 417 (1976); P.E. Belchetz, I.P. Braidman, J.C.W. Crawley, and G. Gregoriadis, *Lancet*, **2**, 116 (1977); G. Gregoriadis, *Nature* (Lodon), **275**, 695 (1978).
3) F. Szoka, Jr., and D. Papahadjopoulos, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 4194 (1978).
4) A.D. Bangham, M.M. Standish, and J.C. Watkins, *J. Mol. Biol.*, **13**, 238 (1965).
5) D.N. Rhodes and C.H. Lea, *Biochem. J.*, **65**, 526 (1957).
6) J. Bieth, B. Spiess, and C.G. Wermuth, *Biochem. Med.*, **11**, 350 (1974).
7) T. Yotsuyanagi, T. Hamada, H. Tomida, and K. Ikeda, *Acta Pharm. Suec.*, **16**, 325 (1979).
8) T. Yotsuyanagi and K. Ikeda, *J. Pharm. Sci.*, **69**, 745 (1980).

[Chem. Pharm. Bull. 29(4)1179—1181(1981)]

The Analgesic Effects of The Decomposition Products of Sulpyrine, N-[2-(5-Hydroxymethyl-2,3,4,5-tetrahydro-2,3,4-trihydroxy)furyl]methyl-N-methylantipyrine and Antipyrinyl-4-peroxide

Sumie Yoshioka, Hiroyasu Ogata,* Toshio Shibazaki, and Akira Ejima

Division of Drugs, National Institute of Hygienic Sciences, 18-1, Kamiyoga 1-chome, Setagaya-ku, Tokyo 158, Japan

(Received September 1, 1980)

The analgesic effects of the decomposition products of sulpyrine, N-[2-(5-hydroxymethyl-2,3,4,5-tetrahydro-2,3,4-trihydroxy)furyl]methyl-N-methylantipyrine (MAAG)and antipyrinyl-4-peroxide (AP), which were reported by us previously, were determined by measurement of the inhibition of writhing caused by the injection of acetic acid into male mice, and compared with the effects of sulpyrine and antipyrine. MAAG and AP showed 62% and 48% of the potency of sulpyrine, respectively, in spite of having hydrophilic substituents. It is suggested that the analgesic effects of these compounds are intrinsic to the compounds themselves.

Keywords——N-[2-(5-hydroxymethyl-2,3,4,5-tetrahydro-2,3,4-trihydroxy)furyl]-methyl-N-methylantipyrine; antipyrinyl-4-peroxide; decomposition product of sulpyrine; analgesic effect; inhibition of writhing syndrome; mouse

Quality control of drugs requires a knowledge of the extent and the products of drug decomposition. In addition, it is very important that the pharmacological and toxicological effects of the decomposition products are characterized.

Sulpyrine, which shows analgesic, antipyretic and anti-inflammatory effects, undergoes hydrolysis in aqueous solution and oxidative degradation both in aqueous solution and in the solid state, as well as undergoing reaction with some additives during preparation,[1-6] and the mode of decomposition of sulpyrine varies according to the reaction conditions. Among the decomposition products of sulpyrine, two previously undetected compounds were found: (i) in injection solutions containing glucose, N-[2-(5-hydroxymethyl]-2,3,4,5-tetrahydro-2,3,4-trihydroxy)furyl]methyl-N-methylantipyrine (MAAG) is formed *via* a condensation reaction, (ii) antipyrinyl-4-peroxide (AP) is produced by oxidative decomposition in the presence of the copper(II) ion.[3,4]

In this report, the analgesic effects of these two decomposition products of sulpyrine are described and compared with those of sulpyrine and antipyrine.

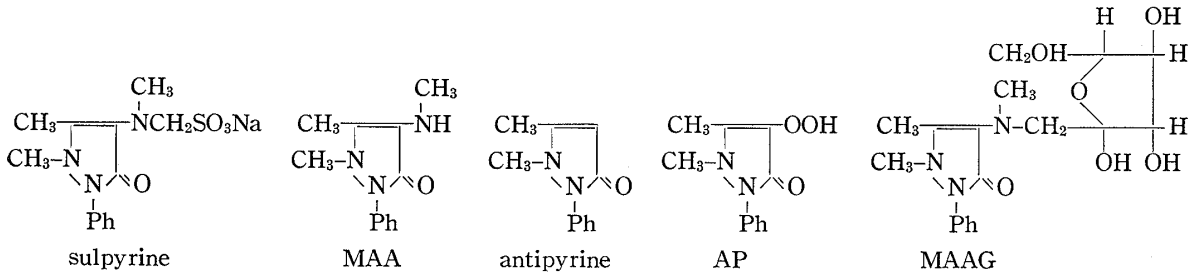

Chart 1

Experimental

Materials——Sulpyrine and antipyrine were of J.P. IX grade. 4-Methylaminoantipyrine (MAA),[7] AP[3] and MAAG[5] were prepared according to the methods described previously.

Determination of Analgesic Potency——Male ddY mice weighing 20—30 g were used. Dose-response curves were obtained by using ten mice at each of three doses. The analgesic potency of each compound was determined by utilizing the inhibition of writhing syndrome caused by acetic acid injection. Solutions of each compound were prepared with isotonic saline solution at three concentrations. The test solution was injected subcutaneously at a volume of 10 ml/kg into mice 10 minutes prior to the intraperitoneal injection of 0.7% acetic acid (10 ml/kg). The total number of stretching episodes was counted from 10 to 20 minutes after acetic acid injection. The mean effect calculated from ten drug-treated animals was compared with that of saline controls. The dose-response curves were calculated by the method of Litchfield and Wilcoxon.[8]

Results and Discussion

Table I shows the analgesic potencies of sulpyrine, its decomposition products and antipyrine, which is a well-known compound with the basic pyrazolone structure. The relative potency of these compounds was represented by taking $1/ED_{50}$ value of sulpyrine as 100, and the value was also corrected for molecular weight. The potency determined by measurement of the inhibition of writhing caused by acetic acid may include a muscle relaxation effect in addition to the analgesic one, but the potencies listed in Table I are probably attributable to the analgesic effect in the concentration ranges tested.

MAA is the main hydrolysis product of sulpyrine,[1,2] and showed the strongest analgesic potency of the products studied. The potency of sulpyrine was 73% of that of MAA. It

Table I. Analgesic Potency

Compound	ED_{50} (mg/kg, *s.c.*)	95% confidence limits	Relative potency ($1/ED_{50}$) (sulpyrine = 100) (mg)	(mol)
Sulpyrine	205	(168—250)	100	100
Antipyrine	128	(103—159)	160	86
MAA	92	(63—133)	223	138
MAAG	355	(293—450)	58	62
AP	263	(202—342)	78	48

is uncertain whether the effect is due to sulpyrine itself. Since sulpyrine decomposes rapidly to MAA in aqueous solution, especially when diluted, it is suggested that the effect of sulpyrine is mainly due to MAA, and that the potency depends on the rate of release of sulpyrine from the depot and on the rate of formation of MAA in the body.

MAAG showed 62% of the potency of sulpyrine. Though MAAG is decomposed further to MAA and aminopyrine, which exhibit high analgesic potencies, the rate of degradation of MAAG is very slow at neutral pH.[5] Thus, the analgesic effect of MAAG is probably intrinsic to the compound itself. The low potency of MAAG may be attributed to its hydrophilicity, which reduces the transfer from the depot to the blood, or reduces the affinity to the sites of pharmacological action.

AP, one of the oxidized products of sulpyrine, also exhibited an analgesic effect though the potency was only 48% of that of sulpyrine, and 56% of that of antipyrine. This compound probably exists as an addition product of water in aqueous solution and is very polar. AP is obtained as a final product under mild oxidative conditions in the presence of a trace of copper(II) ions at pH 5 at 30°, though on heating under acidic conditions it gives 4-hydroxyantipyrine.[3,4] Thus, the analgesic effect of AP is probably intrinsic to the compound itself, and its low potency may be partially due to the hydrophilic properties.

It is very interesting that MAAG and AP, the decomposition products of sulpyrine, have considerable analgesic potencies as determined by the inhibition of writhing caused by acetic acid (their potencies are 62% and 48% of that of sulpyrine, respectively) in spite of having hydrophilic substituents.

Acknowledgement We are grateful to Dr. J. Takahashi, Division of Pharmacology of this Institute, for his helpful advice.

References and Notes

1) S. Yoshioka, H. Ogata, T. Shibazaki, and T. Inoue, *Chem. Pharm. Bull.*, **25**, 475 (1977).
2) S. Yoshioka, H. Ogata, T. Shibazaki, and T. Inoue, *Chem. Pharm. Bull.*, **25**, 484 (1977).
3) S. Yoshioka, H. Ogata, T. Shibazaki, and A. Ejima, *Arch. Pharm.*, **312**, 81 (1979).
4) S. Yoshioka, H. Ogata, T. Shibazaki, and A. Ejima, *Chem. Pharm. Bull.*, **26**, 2723 (1978).
5) S. Yoshioka, H. Ogata, T. Shibazaki, and A. Ejima, *Chem. Pharm. Bull.*, **27**, 907 (1979).
6) S. Yoshioka, H. Ogata, T. Shibazaki, and A. Ejima, *Chem. Pharm. Bull.*, **27**, 2363 (1979).
7) S. Ono, R. Onishi, and K. Kawamura, *Yakugaku Zasshi*, **86**, 11 (1966).
8) J.T. Litchfield and F. Wilcoxon, *J. Pharmacol.*, **96**, 99 (1949).