and 10 were restrained for 24 hours before subcutaneous injection of the hypertonic solutions.

The injection sites of the hypertonic NaCl or urea solutions showed no evident sign of damage beyond transient edema in the control rats. The same was true of those treated with such severe stressors as just-sublethal doses of epinephrine or norepinephrine. Even spinal cord transection conducive to complete paralysis of the hind quarters or prolonged immobilization on a board caused only occasional small patches of cutaneous necrosis at the sites where hypertonic NaCl was applied. On the other hand, serotonin and histamine (two mast-cell products) as well as polymyxin, compound 48/80, dextran, and dextrin (all potent mast-cell dischargers) produced extensive topical necrosis, with thrombosis and hemorrhage in and around the necrotic area, within a few hours after injection (Fig. 1). On the following day, the surface layer of the affected skin region showed signs of total disintegration with exulceration. This response involved virtually the whole area treated with NaCl or urea, respectively; only in the two groups given histamine was this reaction less pronounced. Heparin, another mast-cell product, was totally ineffective in this respect although in some animals it caused minor hemorrhages at the sites where the hypertonic solutions were applied.

Table 1. Effect of various systemic treatments upon the ability of hypertonic NaCl and urea solutions to elicit topical necrosis at subcutaneous injection sites.

Groups	Systemic treatment*	Necrosis at site of topical treatment with	
		NaCl	Urea
1,2	None	0	0
3,4	Epinephrine bitartrate (1 mg s.c.)	0	0
5,6	Norepinephrine bitartrate (1 mg s.c.)	0	0
7,8	Spinal cord transection (by thermocautery above 1st lumbar vertebra)	+	0
9,10	Restraint (24 hr on a board just prior to topical treatment)	+	0
11,12	Heparin (1 mg i.v.)	0	0
13,14	Serotonin creatinine sulfate (2 mg s.c.)	+++	+++
15,16	Histamine phosphate (30 mg i.v.)	++	++
17,18	Polymyxin-B sulfate (2 mg s.c.)	+++	+++
19,20	Compound 48/80 (1 mg s.c.)	+++	+++
21,22	Dextran (60 mg i.v.)	+++	+++
23,24	Dextrin (250 mg i.v.)	+++	+++

* All subcutaneous (s.c.) injections given in 0.2 ml water on belly, all intravenous (i.v.) injections in 1 ml water into jugular vein with the animal under light ether anesthesia.

It may be significant that the above mentioned mast-cell dischargers and mast-cell products (again with the notable exception of heparin and unlike nonspecific stressors) are also potent elicitors of the tissue calcification in the phenomenon of "mastocalcergy" (*1*). A recent review of the literature on mast cells lists more than 30 theories concerning their function (*2*). It would be premature to speculate upon the intimate mechanism of the phenomenon just described, but it appears justified to conclude that close relationships exist between mast cells and tissue resistance to necrotizing agents.

HANS SELYE
Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal, Quebec

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Actinomycete: Isolation and Identification of Agent Responsible for Musty Odors

Abstract. *A compound produced by certain actinomycete cultures is responsible for a persistent musty odor. It has been isolated in high purity and identified by chemical and spectroscopic properties. Possible structures are discussed.*

The musty odor of old potato bins, damp cellars, aged straw piles, stagnant ponds, and so forth, may be attributed in large part to the growth of actinomycetes. Although their normal habitat is soil, these organisms grow abundantly on most types of biological materials. They have in some instances adapted themselves to aquatic environments and have long been known to render potable water supplies nonpalatable by imparting musty and earthy tastes and odors (*1*). Simple amines, short chained aldehydes, and saturated fatty acids have been found in these organisms (*2*). Romano and Safferman (*3*) grew *Streptomyces griseoluteus* and obtained an unidentified odoriferous material which gave a characteristic odor even at a dilution of one to a million. Gaines and Collins (*4*) found that *S. odorifer* produced several simple compounds such as acetic acid, acetaldehyde, ethyl alcohol, isobutyl alcohol, isobutyl acetate, and ammonia. We have cultured certain types of actinomycetes and isolated a unique chemical substance which we believe to be responsible for a very persistent musty odor.

In the spring and summer of 1961 the water of the Cedar River in Iowa had a severe musty taste and odor. At that time Morris (*5*) isolated two genera of actinomycetes (*Micromonospora* and *Streptomyces*) from the river. The predominating *Streptomyces* were later cultured in mass quantities, and Morris *et al.* (*6*) isolated a chemically neutral fraction which contained a musty-odor compound. Briefly, their method consisted of an initial steam distillation that was followed by solvent extraction of the distillates. The neutral fraction from gas chromatography consisted of seven detectable components (Fig. 1). By peak-area analysis, one peak represented a concentration of more than 95 percent of the sample. Because of the low yield of the neutral fraction (approximately 2 g per 500 liters of culture) and the capacity of this material to produce musty odors in extremely high dilutions, the component represented by this peak was considered to be the musty compound. Further attempts at purification of this fraction by microdistillation (1 cm path) at 0.3-mm pressure did not significantly remove all of the impurities represented by the minor peaks in Fig. 1. However, this distillation method did produce approximately 500 mg of a clear, colorless liquid having a specific gravity of 0.987 and an index of refraction (N_D^{25}) of 1.5022, both at 25°C. The N_D^{25} of the neutral fraction before vacuum distillation was 1.5001. In view of this slight change in refractive index after vacuum distillation and the observance of no additional components by modified gas-chromatography procedures, we concluded that this colorless distillate

was sufficiently pure for subsequent molecular and structural analyses.

The empirical formula is $(C_6H_9O)_n$. Ebulloscopic determinations for the molecular weight gave unsatisfactory results. However, the integration of the nuclear magnetic resonance (NMR) spectrum provided the information that the molecule contained 18 protons, hence a molecular formula of $C_{12}H_{18}O_2$ for the musty-smelling compound. The molecular weight of 194 was confirmed by the mass spectrograph. Nuclear magnetic resonance data were obtained for this compound (Table 1). The ultraviolet spectrum (10^{-4} molar, 95 percent ethanol) λ max (ϵ) was 225 mμ (3300) and 301 mμ (8200). The infrared spectrum (neat, wavelength in microns) was 3.38, 5.84, 6.06, 6.30, 6.8, 7.2, 7.3, 8.94, 9.38, 9.77, 10.15, 12.27.

A complete characterization of the structure of this substance is not possible with the data in hand. However, there are certain structural features required by these data, and other structural implications which should be noted.

The molecular formula requires four degrees of unsaturation (double bonds, rings). A carbonyl group accounts for one unsaturation and from the bond at 5.84 μ (*7*) in the infrared spectrum is a five-ring (or unusually strained six-ring) α,β-unsaturated ketone, an ester, or an aliphatic ketone. The ester (or lactone) is eliminated by a negative result in the hydroxamic acid test (*8*). A conjugated system is required by the ultraviolet spectrum (*9*). The NMR spectrum requires two *cis*-vinyl protons for the AB quartet at 5.86–6.96 parts per million and the chemical shift 6.96 requires that this vinyl proton be beta to a carbonyl group (*9*). Collectively this evidence requires the $-CH=CH-C=O$ moiety in a five-membered ring (or larger strained ring). The carbonyl group cannot be an aldehyde: the aldehyde proton regions in the IR and NMR spectra show no absorption.

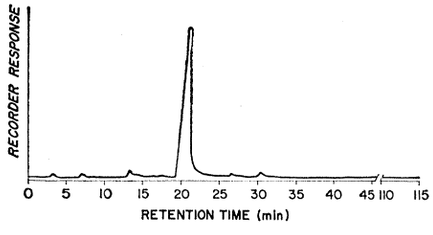

Fig. 1. Gas chromatogram of 0.2 μl of the actinomycete musty material. Sensitivity 4 ×; 50-m capillary coated with Perkin-Elmer Ucon-oil LB-550 ×. Temperature of column 150°C, isothermal. Helium pressure, 2 atm.

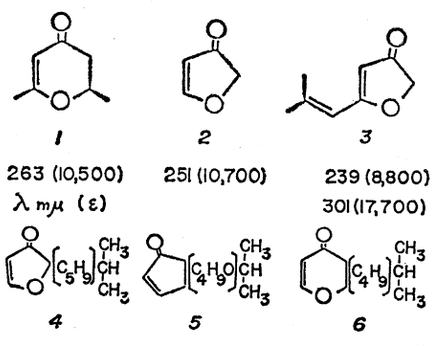

Fig. 2. Structures 1 to 6.

Table 1. Nuclear magnetic resonance spectrum of compound from actinomycetes. (Varian A-60, TMS internal, neat)

Chemical shift, δ (ppm)	Integrated intensity*	Splitting, J, (cy/sec)	Multiplet pattern
0.9	6	6	Doublet } A_6B isopropyl
1.9	1	6	Septet (obscured) }
1.23	2	0	Singlet Aliphatic
1.23	2	15	} A_2B_2 quartet, geminal coupling
2.3	2	15	
2.25	1	7.5	} AB quartet, vicinal aliphatic
2.6	1	7.5	
5.86	1	7	} AB quartet, *cis*-vinyl
6.96	1	7	

* Although the 18th proton does not appear as a resolved signal, the integration of the NMR spectrum allows its presence in the 2 ppm region.

The NMR spectrum shows an A_6B pattern (0.9, 1.9 ppm) typical of the isopropyl group. The isopropyl group is also evident in the 7-micron region of the infrared spectrum and in the peak of the mass spectrum where the ratio of mass to charge is 43. Examination of the NMR spectrum reveals no other resonance signal for a methyl group, hence the isopropyl group is the only chain terminus. All other protons must be bound to carbon atoms internal to rings or chains.

Three degrees of unsaturation are accounted for in the cyclic unsaturated ketone moiety. The fourth degree of unsaturation required by the molecular formula cannot be a double bond. The only vinyl protons evident in the NMR are accounted for. Any structure drawn containing a second carbon-carbon double bond requires two chain termini. Only one terminus is allowed by NMR, hence the fourth degree must be a ring in addition to the ring containing the unsaturated ketone moiety.

The ultraviolet spectrum is difficult to reconcile with structures allowed by the data above. The maximum conjugation available is the $-O-C=C-C=O$ system which, as the model compounds 1 to 3 show (*10*), is not adequate to explain the intense band at 301 mμ (Fig. 2). The extended conjugation of structure 3 is not available to the compound in question. The data suggest structure 4 as the partial structure best representing the compound. The ultraviolet spectrum cannot be reconciled until the entire structure is known, hence possibilities such as structures 5 and 6 cannot be rejected entirely. The influence of the bracketed moiety upon the properties of the molecule remains unknown.

The partial structures are useful for considering further chemical degradation studies and selecting possible treatment procedures for improving the taste and odor of water polluted by this actinomycete metabolite.

JOHN D. DOUGHERTY
State Hygienic Laboratory,
University of Iowa, Iowa City
RICHARD D. CAMPBELL
Department of Chemistry
ROBERT L. MORRIS
State Hygienic Laboratory

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