

Isopropylidengrayanotoxin-II. Colourless grains from *n*-hexane-chloroform mixture, mp 164—164.5°. *Anal.* Calcd. for C₂₃H₃₆O₅: C, 70.37; H, 9.24. Found: C, 70.54; H, 9.42.

20-Nor-10-ketoisopropylidengrayanotoxin-II was obtained from isopropylidengrayanotoxin-II by ozonolysis. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3400, 1695. NMR $\delta_{\text{ppm}}^{\text{CDCl}_3}$: 0.95 (3H, s), 1.12 (3H, s), 1.35 (6H, s), 1.40 (3H, s), 3.41 (1H, br-s), 3.61 (1H, m), 4.46 (1H, m). ORD (in MeOH, *c* = 0.193): $[\phi]_{313.4}^{\text{trough}} - 6230^\circ$, $[\phi]_{272}^{\text{peak}} + 6070^\circ$.

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Measurements of Extra-Weak Chemiluminescence

It has been reported recently that the transfer of electronic excitation energy by resonance between proteins and carcinogenic aromatic compounds¹⁾ or non-steroidal anti-inflammatory drugs²⁾ may play an important role in their biological activities, and that some nucleophilic reactions in the first electronic excited state of substrate seem to take part in the mechanism of hydroxylation reaction of aromatic substrates by monooxygenase.³⁾

These results suggest that some biological constituents are in their electronic excited state at some stage in biochemical processes. However, is it possible that the electronic excited state of molecules such as aromatic compounds is produced in a dark cell without light? As a reasonable answer to this question, the extra-weak spontaneous bioluminescence from living organisms may be considered. Gurwitsch, *et al.*⁴⁾ investigated "the mitogenetic radiation," which is the spontaneous emission of ultraviolet ray from various biological objects such as excited nerves, muscles, and so on, by using the so-called biological detectors. Konev, *et al.*⁵⁾ observed a very weak spontaneous luminescence in the ultraviolet and visible region from living organisms by means of a kind of geiger counter. However, these methods are not reproducible, and the results of their determinations seem to be under criticism.

The experiment reported is of a preliminary nature prior to attempting the more difficult observation of the

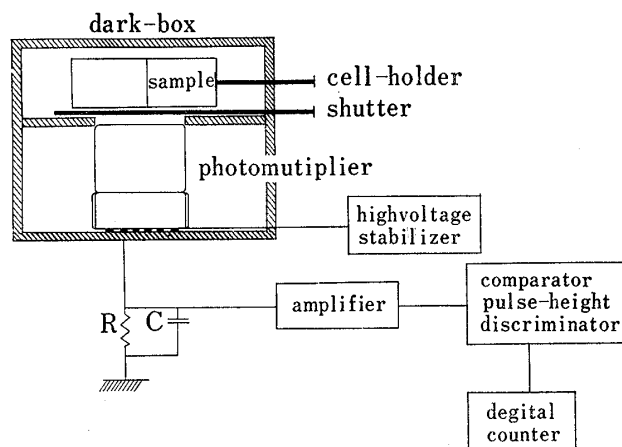


Fig. 1. A Simplified Schematic Representation of the Apparatus

- 1) K. Mizuno, S. Hata, and S. Tomioka, *GANN*, **60**, 469 (1969).
- 2) K. Mizuno, S. Hata, and S. Tomioka, *Chem. Pharm. Bull.* (Tokyo), **18**, 186 (1970).
- 3) K. Kumaki, S. Hata, K. Mizuno, and S. Tomioka, *Chem. Pharm. Bull.* (Tokyo), **17**, 1751 (1969).
- 4) A.G. Gurwitsch and L.D. Gurwitsch, "Die Mitogenetische Strahlung," Veb Gustav Fischer Verlag, Jena, 1959.
- 5) N.A. Troiiskii, S.V. Konev, and M.A. Katibnikov, *Biofizika*, **6**, 238 (1961).

TABLE I. Photon Counting Values of Extra-Weak Chemiluminescence^{a)}

| | Noise ^{b)} (count/10 sec) | Noise+signal ^{c)} (count/10 sec) |
|--|---------------------------------------|--|
| 1) ^{d)} 8% glycine aq. soln. (50 ml) +30% H ₂ O ₂ aq. soln. (3 ml) | 1160 | 2126 |
| | 1100 | 2102 |
| | 1275 | 1983 |
| | 1161 | 2116 |
| | 1158 | 2112 |
| | 1171 (average) | 2008 (average) |
| 2) ^{e)} Ergosterol (160 mg)+linoleic acid (4 ml) | 992 | 3431 |
| | 888 | 3444 |
| | 923 | 3416 |
| | 951 | 3471 |
| | 938 | 3419 |
| | 938 (average) | 3436 (average) |
| 3) ^{f)} 0.2% KMnO ₄ aq. soln. (4 ml) +6% oxalic acid aq. soln. (2 drops) | 1983 | 2428 |
| | 1979 | 2417 |
| | 2053 | 2516 |
| | 1943 | 2441 |
| | 2098 | 2502 |
| | 2011 (average) | 2461 (average) |

a) It is impossible to measure these luminescences with a Shimazu GSF-16Type Spectrofluorometer and to observe them with a naked eye even in a completely dark room.

b) dark current of photomultiplier without sample

c) photo current by sample

d) glycine: a G.R. grade purchased from the Tokyo Chemical Ind. Co., Ltd.

H₂O₂: a G.R. grade purchased from the Wako Pure Chemical Ind. Co., Ltd.

e) Ergosterol: a G.R. grade purchased from the Tokyo Chemical Ind. Co., Ltd.

linoleic acid: a E.P. grade purchased from Tokyo Chemical Ind. Co., Ltd.

f) KMnO₄: a G.R. grade purchased from Wako Pure Chemical Ind. Co., Ltd.

oxalic acid: a E.P. grade purchased from Tokyo Chemical Ind. Co., Ltd.

mitogenetic radiation which, if successful, will yield valuable information on various biological reactions. Using the photon counting method indicated in Fig. 1, we have observed some extra-weak chemiluminescences as a model of mitogenetic radiation. The experiments gave positive results as indicated in Table I and individual oxidizer or substrate is counted as same level as the dark current. In the second column of Table I are given the noise-level which is represented by the dark current of photomultiplier in a 10 sec interval; third column is the count in the same interval where the extra-weak chemiluminescences were expected. Hence, the differences between them are a measure of the signal which is due to the extra-weak chemiluminescence of samples.

All the samples were measured with HTV-R 329 end-on type photomultiplier (the wavelength of maximum response is 3850 Å) at 25°. A quartz vessel 10 mm in length was filled with the sample solution and set in front of the window of photomultiplier. Measurements were begun a few minutes after preparation of the samples. Although it is necessary to succeed further investigation, it will be assumed that the extra-weak spontaneous bioluminescence from various living organisms can be measured by such a technique.

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