ages. It seems improbable that completely different materials (shells and inorganic phosphate nodules) should show the same very positive isotopic compositions by chance or as a result of unknown postdepositional processes. Thus we may reasonably consider the most positive data from Miocene fossils to result from good preservation of the original isotopic composition of the phosphate precipitated in isotopically positive waters.

The hypothesis proposed to interpret the results from Mesozoic and Tertiary fossils seems rather difficult to accept because it changes widely our ideas of the isotopic history of the oceans and of the rate of the isotopic-exchange processes affecting fossils throughout geologic time. The supposed variation of the oxygen-isotope composition of ocean water [about 5 to 7 per mille (*8*) during about 2×10^8 years] is very great but does not seem unbelievable (*2*). The sedimentation (either chemical or biologic) of carbonate, phosphate, sulfate, silicate, and such materials may be considered responsible for such variation, because of the enrichment in oxygen-18 of the different chemical compounds relative to ocean water. We must admit that acceptance may be difficult of the hypothesis proposed to explain our $\delta O^{18}(PO_4^{3-})$ results, but we assume that there are no serious arguments for rejection.

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8. These values can be approximately evaluated if one assumes an average temperature of 15° to 20°C for the Jurassic ocean and calculates the δ-difference on the basis of the most positive $\delta O^{18}(PO_4^{3-})$ obtained from Jurassic belemnites.
9. Supported by C.N.R.–C.N.E.N. contract 115-1159-1472 and by NSF grant GA-351. We thank G. Giaconi and P. Perusini for assistance.

13 March 1968

Photolytic Cleavage of Sulfonamide Bonds

Abstract. *It has been found that the sulfonamide bond is relatively susceptible to photolytic cleavage. The breakdown was effected either by irradiation with a source having a continuous emission above the wavelengths of 1800 angstroms or by another source emitting principally at 2537 angstroms. Less destruction of the amino acids was seen with the latter relative to the sulfonamide bond cleavage. The cleavage was not effected by irradiation at wavelengths greater than about 3000 angstroms. Side reactions were noted involving decarboxylation, demination, and destruction of certain susceptible amino acids such as tryptophan. In only one case was a product found that arose from cleavage of a carboxamide bond; glycyltyrosine gave glycine and tyrosine upon irradiation. A yield of 75 percent of the corresponding amino acid has been obtained by irradiation of tosylhistidine; yields of 75 to 100 percent have been obtained from sulfamic acid (NH_2SO_3H). A qualitative method for identifying sulfonylated amino acids is described.*

We report here some observations on the effect of ultraviolet light on compounds containing the partial structure —SO_2NH—. We have found that the sulfonamide bond is susceptible to photolytic attack under conditions where many other bonds in organic molecules are attacked to a minor degree (*1*). In particular, the photolytic S—N bond cleavage in the sulfonylated amino acid and peptide derivatives is the change most in evidence for all of the amino acid derivatives examined except for the derivatives of cystine, cysteine, and tryptophan. Although the yields of the released amino acid are not yet high enough to make the process a serious contender for deblocking, say, tosylated amino acids, we do not feel that the highest yields have yet been attained. Aside from the potential use in the area of peptide synthesis, the cleavage is intrinsically interesting because of the relative ease with which this cleavage may be effected.

Historically the removal of a tosyl group (*p*-toluenesulfonyl-) from tosylated amino acids and peptides can be achieved without appreciable cleavage of the peptide bonds by treatment of these derivatives with hydrogen iodide (*2*). Cleavage of sulfonamide bonds by anhydrous hydrogen bromide (*3*) has been extended successfully to peptide derivatives (*4*). Treatment with sodium in liquid ammonia provided a generally applicable deblocking procedure (*5*). The photolysis of the appropriate amino acid and peptide derivatives may be a potential new method of removing a blocking group commonly used in peptide synthesis.

The ultraviolet energy sources used were a Hanovia utility quartz lamp (Hanovia) and a germicidal lamp (General Electric, 25 watt, G25T8). The former has a continuous spectrum from 1800 Å through the visible; the latter gives a sharp line at 2537 Å and relatively little else except in the visible. Some of the quantitative determinations of the free amino acids formed from the derivatives are shown in Table 1. Solutions of tosyl amino acids (1 ml in volume) were kept under mineral oil during exposure to ultraviolet. The exposures were carried out in transparent quartz test tubes (1.5 by 10 cm) (Fig. 1) situated at a distance of 1.5 cm from the Hanovia lamp. The temperature of the solution was maintained at the desired level by using a "cold finger" probe (Fig. 1). The optimum time was used in obtaining the yields shown in Table 1, since the released amino acid

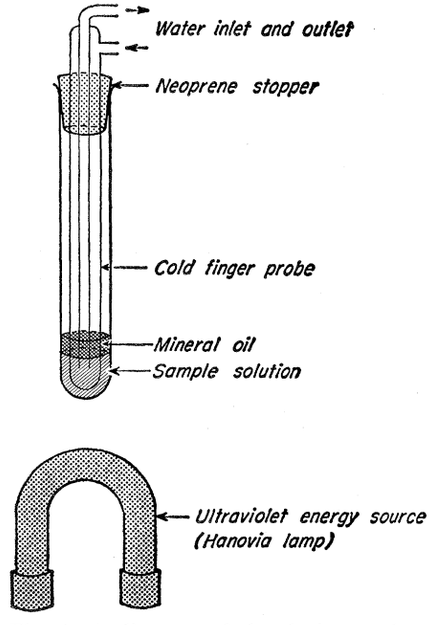

Fig. 1. A diagram of the device used to provide a controlled temperature during irradiation with the Hanovia lamp. Water, or a glycerol-water mixture, was pumped through the "cold finger" probe from a constant temperature bath.

Table 1. Yields of amino acids from *p*-toluenesulfonyl derivatives at various temperatures. Abbreviations: TsLeu, tosylleucine; TsHis, tosylhistidine; TsArg, tosylarginine; TsCys, tosylcystine.

Sample*	Concentration (mg/ml)	Temperature of exposure (°C)	Time for maximum yield (hours)	Yield (% of theoretical)†
TsLeu	2.444	25	3	22 ± 1
TsLeu	2.444	50	2	21 ± 1
TsLeu	2.444	90	1	48 ± 0
TsHis	5.344	25	4	46 ± 2
TsHis	5.344	50	4	48 ± 0
TsHis	5.344	90	3	57 ± 2
TsArg	2.348	25	2	31 ± 1
TsArg	2.348	50	1	31 ± 1
TsArg	2.348	90	0.5	31 ± 1
TsCys	3.71	25	4	29 ± 2
TsCys	3.71	50	4	31 ± 4
TsCys	3.71	90	3	41 ± 0

* Dissolved in water. † Corrected yield measured and calculated by the method of Moore and Stein (*6*).

is destroyed and excessive irradiation reduces the yields significantly. No measurable breakdown of tosylated amino acids was observed when the samples were contained in new pyrex tubes; thus it may be concluded that irradiation at wavelengths of greater than about 3000 Å is ineffective.

The production of the amino acids is exponential in nature, as shown by the release of leucine from methanesulfonylleucine (Fig. 2). At longer exposure times the destruction of the amino acids becomes appreciable and this, at least in part, after the blocking group has been destroyed. The photochemical change of the amino acid includes the deamination of the free amino acid as shown by the release of free ammonia. Decarboxylation was also evident from the appearance of products tentatively identified as the amines corresponding to the amino acids. The rate of production and yield increase with decreasing concentrations; a 75 percent yield of histidine from tosylhistidine has been achieved at concentration of 1.55 mg/ml of tosylhistidine.

Sulfamic acid (NH_2SO_3H) upon irradiation gave ammonia in yields of 75 to 100 percent, which is higher than for the release of any amino acid. It could be shown that the maximum yields of the free amino acid increase with an increase in the temperature of exposure. The maximum yields at 25°C are exceeded by 120, 40, 0, and 28 percent, respectively, by irradiating tosylated leucine, tosylated histidine, tosylated arginine, and tosylated cystine at 90°C.

Twelve amino acid and peptide derivatives were exposed to the Hanovia lamp on chromatographic papers. These derivatives were spotted on chromatographic papers which were then exposed to the ultraviolet light source. The distance of the samples from the lamp was 6.5 cm, and the cooling was done by passing a stream of air over the paper during the entire period of exposure; the temperature was less than 34°C in every case. The control samples were then applied and the chromatograms were developed by using conventional methods.

During the course of these experiments it was noted that under controlled conditions of temperature the chromatographic papers can withstand the radiation from either of the ultraviolet lamps, thus no interference with the test material was expected. Some of the derivatives tested successfully were benzenesulfonyl, *p*-toluenesulfonyl, α- and β-naphthalenesulfonyl and methanesulfonyl derivatives. In all cases ninhydrin-positive products corresponding to the free amino acids and peptides were obtained, indicating that the photodynamic effect is due to the absorption of ultraviolet light energy by the sulfonamide linkage. Support for the concept that the sulfonamide bond itself was susceptible to the photolytic cleavage does not rest only on the fact that yields of amino acids and peptides were not influenced by the R group of R—SO_2NH—. When R = OH (sulfamic acid) the best yields were found presumably because of the smaller number of ways the reactants and products can be destroyed.

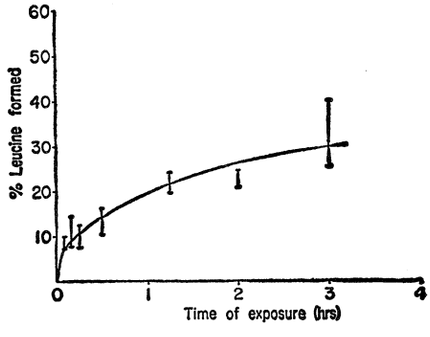

Fig. 2. Yield of leucine formed from methanesulfonylleucine at 25°C as a function of time during irradiation in 0.002*N* NaOH, at a concentration of 1.466 mg/ml.

The ease with which the release of amino acids and peptides can be effected is the basis for a new method for identifying less than microgram quantities of sulfonylated amino acids and peptides. For example, the sulfonylated amino acid or peptide was developed chromatographically in one dimension; this was followed by irradiation and development in a second dimension. The released peptide or amino acid was then visualized with a suitable color reagent. Thus, sulfonylated amino acids may be separated from chemically different substances in one dimension, irradiated, and then identified by chromatography in the second dimension.

Some *p*H-dependence was noted. Exposure of tosylhistidine was carried out at *p*H values of less than 1 to more than 13 in water. The color yield decreases at basic *p*H values as compared to that in the neutral or acidic solutions. A stronger positive test for ammonia was obtained in the basic and acidic solutions than was seen in the neutral solutions.

In summary, it has been established that a variety of sulfonylated amino acid and peptide derivatives can be "deblocked" by ultraviolet irradiation under conditions neither effecting comparable cleavage of carboxamide bonds nor causing excessive destruction of most of the amino acids.

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1 March 1968