(IV) in 76% yield,[9] which was identified by direct comparison with an authentic sample.[6] The formation of IIIa and IV can be interpreted in terms of the diaziridine intermediate (V) (through path b), which was also postulated in the photoisomerization of N-acyliminopyridinium betaines to the corresponding diazepine derivatives (through path a). So far only two examples which follow the path b are known[10]; N-carbethoxyimino-3,5-dimethylpyridinium betaine[1b] and N-acetyliminoquinolinium betaine.[4]

In contrast to the photochemical transformation, thermolysis of neat Ia at 190—200° results in fragmentation to isoquinoline (80%), benzanilide (VI) (29%), diphenylurea (VII) (trace) and benzamide (VIII) (trace). Similarly the betaine (II) decomposed at 190—200° to give quinoline (60%), VI (45%), VII (trace) and VIII (trace). These results are again contrasted with the case of N-benzoyliminopyridinium betaine,[2] which, upon heating at 190—200°, gave pyridine (27%) and VII (30%) as the major fragmentation products.

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9) T. Shiba, Y. Yamane, and H. Kato,[4] reported that in the photolysis of II the fragmentation to quinoline and nitrene derivative was the main reaction path and no isomeric product could be isolated.
10) Irradiation of N-carbethoxyiminoisoquinolinium betaine (Ib, mp 129—130°) in ethanol and N-acetyliminoisoquinolinium betaine (Ic, hygroscopic crystals) in methanol gave 1-carbethoxyaminoisoquinoline (IIIb)[11] and 1-acetamidoisoquinoline (IIIc)[12] in 62 and 60% yield, respectively.
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The Interactions between Bisulfite and Amino Acids. The Formation of Methionine Sulfoxide from Methionine in the Presence of Oxygen

Recent investigation has shown that bisulfite reacts with a variety of nucleic acid bases: (1) By an ionic mechanism bisulfite adds to the 5,6-double bond of pyrimidine nucleosides,[1,2] and (2) by a free radical chain mechanism it reacts with 4-thiouridine[2] and with N^6-Δ^2-isopentenyladenosine.[3] Mutagenic activity of bisulfite has also been demonstrated using phage[4] and bacteria.[5] These findings have implied a possible genetic hazard of environmetal bisulfite to higher living organisms.

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We have now surveyed the interactions of bisulfite with amino acids. Each amino acid was kept at pH 7 and room temperature, (*a*) in 1 M bisulfite solution, and (*b*) in 0.1 M bisulfite solution with oxygen-bubbling. One may expect that an ionic reaction can be detected by the condition *a* and a free radical reaction by the condition *b*.[2] After 1 hr of the reaction, ninhydrin-positive products were detected by paper chromatography using the solvent systems; (i) butanol-acetic acid-water (4:1:5); (ii) water-saturated phenol; (iii) iso-amylalcohol-pyridine-water (35:35:30); (iv) phenol-*m*-cresol-25 mM borate, pH 9.5 (25:25:7); (v) butanol-water (86:14). Cysteine and cystine have not been tested because the cystine formation from cysteine by oxygen, as well as the sulfitolysis of cystine, has been well documented.[6] Amino acids tested were leucine, serine, threonine, proline, lysine, arginine, glutamic acid, glutamine, tyrosine, tryptophan, histidine, and methionine.

Products other than the starting material were observed only with tryptophan and methionine. The transformation of tryptophan was detectable only in the condition *a* but not in *b*. This reaction was slow and obviously complex, giving at least three products. Some properties of the products are summarized in Table I. Elucidation of the structures of these products awaits further experiments. Tryptophan has been reported to yield a complex mixture of compounds on treatment with bisulfite at 100° for 20 hr.[7] The structures of some of the

Table I. Properties of the Products derived from Tryptophan

Compound	*Rf* in solvent i	UV in H_2O λ_{max}	UV in H_2O λ_{min}	Color with ninhydrin reagent	Color with *p*-dimethyl-amino benzaldehyde reagent	Ionic character as determined by paper electrophoresis at pH 7	Incorporation of ^{35}S when ^{35}S-bisulfite was used as the reagent
Tryptophan	0.51	278 287.5	242 285	purple	violet	neutral	−
Product 1	0.42	255 288 (fluorescent)	248 278	purple	orange	neutral	−
Product 2	0.18	273	245	purple	orange	anionic	+
Product 3	0.16	258 ~290 (sh) (fluorescent)	248	purple	orange	anionic	+

Table II. Identification of Methionine Sulfoxide with the Reaction Product formed from Methionine

	Rf values					Mobility (cm) in electrophoresis toward the cathode	
	Solvent i	ii	iii	iv	v	pH 1.5[a]	pH 7.0[b]
Product	0.09	0.77	0.05	0.65	0	5.0	0
Methionine sulfoxide[8]	0.10	0.77	0.05	0.65	0	5.0	0
Methionine sulfone[c]	0.11	0.59	—	0.27	—	—	—
Methionine	0.36	0.77	0.29	0.65	0.18	5.4	0

a) 85% HCOOH-AcOH-H_2O (5:15:80), 70 v/cm, 15 min
b) 25 mM phosphate buffer, 70 v/cm, 15 min
c) E. Schram, S. Moore and E. J. Bigwood, *Biochem. J.*, **57**, 33 (1954)

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products have been established. None of them, however, corresponds to the product 1, 2 or 3 in Table I.

Both in conditions *a* and *b* methionine reacted rapidly, giving a single product, which was identified to be methionine sulfoxide by comparison with an authentic sample[8] (Table II). This reaction required the presence of both oxygen and bisulfite. Hydroquinone (2 mM) in 0.1 M bisulfite inhibits the reaction almost completely. This suggests that the reaction involves a free radical chain mechanism.[9] By paper chromatographic fractionation and subsequent determination of the amino acids with ninhydrin,[10] the progress of the reaction was measured. The amount of methionine sulfoxide plus methionine recovered from the chromatogram accounted for 92% of the methionine used as the starting material. The results are presented in Fig. 1. At three different levels bisulfite concentration, the reaction was rapid in its initial stage, but it leveled of after a certain time. This phenomenon can be explained in terms of a rapid break down of bisulfite by aerobic oxidation. It can also be seen that the efficiency with which bisulfite is utilized for the sulfoxide formation is higher at a more dilute bisulfite concentration. Thus, from 20 mM methionine 4 mM sulfoxide was formed in the 10 mM bisulfite solution, while only 12 mM sulfoxide was produced in the 50 mM bisulfite solution. Indeed, repeated addition of fresh bisulfite, 10 mM at a time, was found to be highly efficient for the sulfoxide formation, and the reaction was completed in about 2 hr.

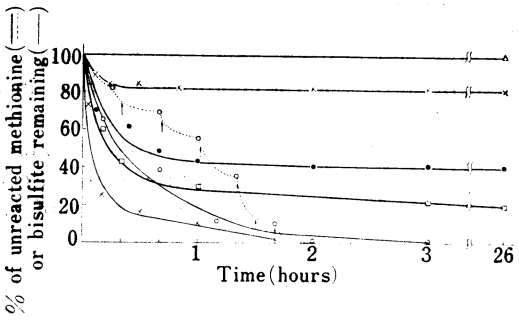

Fig. 1. Effect of Bisulfite Concentration on Methionine Sulfoxide Formation

The reaction was carried out in 0.4M sodium phosphate buffer, pH 6.9. The total volume was 30 ml. The reaction was started by addition of bisulfite, and allowed to proceed at room temperature under mechanical stirring while air was being bubbled through the solution at a rate of 8 ml/sec. Aliquots (5 μl each) were taken and applied on Toyo filter paper No. 51A. After chromatogaphy in solvent i the amino acids were eluted with water and their quantities were determined. The experiment with repetitive addition of bisulfite was performed in a scale 1/3 of that of the above reaction. At each addition, 0.1 ml of 1M bisulfite, pH 6.9, was dropped into the reaction mixture. Determination of the bisulfite content by iodine titration was carried out for solutions omitting methionine from the above reaction mixture, since methionine is known to consume iodine.[12]

	Methionine	Bisulfite
—△—	20 mM	omitted
—×—	20 mM	10 mM
—●—	20 mM	50 mM
—□—	20 mM	100 mM
—x̊—	omitted	10 mM
—○—	omitted	50 mM
---○---	20 mM	10 mM, the arrow indicating each addition of fresh bisulfite

The aerobic oxidation of bisulfite has been recognized to be a free radical chain process.[11] In this process various radicals having oxidizing power, for example $\cdot HO_2$,[9] have been assumed to be generated. The methionine sulfoxide formation may well be brought about by the action of such oxidizing radicals. The higher effectiveness of a more dilute bisulfite solution may be explained in terms of a competition between methionine and bisulfite in utilizing the oxidizing agent. Several methods are known to convert methionine into its sulfoxide.[12] The present finding has provided a new such procedure. It must be added that while this manuscript was in preparation, Yang has reported a similar sulfoxide formation from methionine in the Mn^{2+}-sulfite-O_2 system.[13]

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Stereostructure of Grayanotoxin VIII, IX, X, and XI. Toxins of *Leucothoe grayana*

From the leaves of *Leucothoe grayana* MAXIMOWICZ (Ericaceae), a famous poisonous shrub in Japan, seven toxic diterpenoids, grayanotoxin I, II, III,[1,2] IV,[3] V,[3,4] VI, and VII,[4] have been isolated. Further survey of the toxic constituents of the leaves has led to the isolation of four novel diterpenoids for which the terms grayanotoxin VIII, IX, X, and XI (G-VIII, G-IX, G-X, and G-XI) are proposed.

G-VIII, $C_{20}H_{30}O_4$, mp 190—193°, was revealed[5] to have two tertiary methyls (1.06, 1.49 ppm), two vinylidenes (1626, 887 cm^{-1}, 4.93—5.11 ppm), and hydroxyls (3350 cm^{-1}) in which three are secondary (3.83, 4.31, 4.39 ppm). On acetylation G-VIII gave the diacetate (V). Analysis of the nuclear magnetic resonance (NMR) spectra of G-VIII and its acetate (V) indicated that the structure of G-VIII resembles that of G-VII (VI).[4] Indeed, the spectral properties of G-VIII can be well rationalized provided that the 15-ene system in G-VII is replaced by a 16-ene system in G-VIII. In order to confirm this assumption, G-II (VII) in dioxane was heated under reflux in the presence of cupper sulfate to yield two dehydration products, one being identified as G-VIII and the other identified as G-VII (VI). On the basis of the above evidence, it is established that G-VIII is represented by formula I.

G-IX, $C_{22}H_{32}O_5$, mp 151—152°, was shown[5] to possess two tertiary methyls (1.01, 1.24 ppm), a vinyl methyl (1.71 ppm), a vinylidene (1625, 891 cm^{-1}, 4.94, 5.08 ppm), a vinyl hydrogen (5.13 ppm), a secondary O-acetyl (1730, 1240 cm^{-1}, 2.06, 5.47 ppm), and hydroxyls (3370 cm^{-1}), two of which are secondary (3.52, 3.63 ppm). Then alkaline hydrolysis of G-IX was carried out to yield deacetyl-G-IX which was identified as G-VII (VI). The C-14 hydrogen signal in the NMR spectrum of G-IX occurs at a lower-field region (5.47 ppm), indicating that the C-14 hydroxyl is acetylated. Therefore, G-IX is concluded to have the stereostructure II.[6]

G-X, $C_{20}H_{32}O_5$, mp 165.5—166.5°, was indicated[5] to have two tertiary methyls (1.00, 1.22 ppm), two vinylidenes (1630, 883 cm^{-1}, 4.87, 4.97, 5.11 ppm), a secondary O-acetyl (1730, 1235 cm^{-1}, 2.05, 5.11 ppm), and hydroxyls (3340 cm^{-1}), two of which are secondary (3.51, 3.61 ppm). G-X was then subjected to alkaline hydrolysis furnishing deacetyl-G-X which was found identical with G-VIII (I). Since the C-14 hydrogen signal in the NMR spectrum of G-X discloses a lower-field shift (5.11 ppm), the C-14 hydroxyl is acetylated. It follows that G-X is shown as III.[6]

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