peaks as shown in figure 2. And also these doublet fragment ions having the same structure but the different mass number should have given a free interval of the m/e values if the paired peaks had not shown a definite ratio. Thus, it is expected to obtain more satisfactory results by using more suitable reagents.

Moreover, this technique will be applied conveniently to analysis of endogenous substances which are difficult to use a conventional 1:1 mixture technique, and also enable to carry out simultaneously qualitative and quantitative analyses in picogram level of endogenous substances with high reliability.

The authors hope the present technique to be used for the investigation of biological substances in the field of clinical pharmacology and biochemistry.

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Received May 17, 1974


Fluorometric Assay of Bisulfite

One of the most commonly used methods for the determination of sulfite or bisulfite is the West-Gaekte method and modified colorimetric methods using acid-bleached parar-
saniline and formaldehyde. Other colorimetric methods such as those with mercuric chloranilate,\textsuperscript{3} 5,5'-dithiobis-(2-nitrobenzoic acid),\textsuperscript{4} iron (III) and 2,4,6-tri(2-pyridyl)1,3,5-triazine,\textsuperscript{5} and iron (III) and 1,10-phenanthroline\textsuperscript{6} have been reported, in addition to spectrophotometric methods by means of ultraviolet absorption.\textsuperscript{4,7} Recently, Axelrod, et al.\textsuperscript{8} recommended a fluorometric method for determination of sulfite primarily based on the quenching of fluorescent 5-aminofluorescein by sulfomethylation with hydroxymethanesulfonic acid derived from sulfite and formaldehyde.

We found that bisulfite reacts with N-(p-dimethylaminophenyl)-1,4-naphthoquinoneimine (indophenol blue, \(\alpha\)-naphthol blue, I) to give blue fluorescence. In the present communication, an fluorometric assay of bisulfite based on the reaction is described.

Formation of the fluorescent product(s) (II) from bisulfite and I was maximum at pH 3.5–4.0 and the reaction was completed at 37\(^\circ\) within 30 min. No fluorescence was observed in a strong acidic or alkaline medium. Fluorescence intensity of II was dependent upon pH; the fluorescence was not observed below at pH 6, but it gradually began to increase at around pH 6.5 to reach a constant value at pH 11. As shown in Fig. 1, the excitation maximum is at 340 nm and fluorescence at 435 nm at pH 13.5 (uncorrected).

Based on all these observations, the following procedure was established for fluorometric determination of bisulfite; 1.0 ml of sample, standard, or blank solution is mixed with 2.0 ml of 0.1M HCl-citrate buffer (pH 3.62). To prevent the oxidation of bisulfite catalyzed by metal ions, 5 \(\mu\) mole of EDTA was preliminarily added to the standard or blank solution, or conversely to the buffer solution in the case of analyzing the sample. Add 1.0 ml of 0.1 mM I in ethanol, mix, and shake at 37\(^\circ\) for 30 min with a 5 cm stroke at 150 strokes per min. Add 1.0 ml of 2N NaOH, mix, and measure the fluorescence by setting the excitation at 340 nm and the fluorescence emission at 435 nm. The lower limit of determination is 1.5\(\times\)10\(^{-8}\) mole and the working curve is linear up to 1.5\(\times\)10\(^{-7}\) mole in this procedure.

Pyrosulfite (\(\text{S}_2\text{O}_3^{2-}\)) and sulfite (\(\text{SO}_3^{2-}\)) gave approximately the same degree of fluorescence intensities by reacting with I as that of bisulfite. Hyposulfite (\(\text{S}_2\text{O}_4^{2-}\)) and sulfide (\(\text{S}^{2-}\)) ions, and reduced glutathione also yielded fluorescent products, the intensities being 40, 17 and 8\% respectively on a molar basis. Other inorganic sulfur compounds (\(\text{SO}_4^{2-}\), \(\text{S}_2\text{O}_3^{2-}\), \(\text{S}_2\text{O}_4^{2-}\), \(\text{S}_4\text{O}_6^{2-}\), \(\text{SCN}^-\)), some reducing agents (\(\text{Fe}^{2+}\), \(\text{Ce}^{3+}\), \(\text{Cu}^+\), \(\text{CN}^-\) and \(\text{L}-\text{ascorbic acid}\)) and organic sulfur compounds including thiols, disulfides, S-sulfonic acids (Bunte salts), sulfides, thioureas, thiurams, xanthates, dithiocarbamates, sulfonic acids, sulfinic acids, sulfamates, isothiocyanates, thiocyanates, sulfates, sulfoxides, and sulfones, gave little or no fluorescence.

\textsuperscript{5} B.G. Stephens and H.A. Sudde, \textit{Analyst}, 95, 70 (1970).
Although the compound which yields fluorescence by reacting with I is not limited to bisulfite as mentioned above, in practical point of view, the present method would permit selective determination of bisulfite in biological materials; because highly interfering compounds, pyrosulfite and hyposulfite, are usually not found in nature, and furthermore, inorganic sulfide and thiols could be eliminated by treating with p-chloromercuribenzoic acid prior to addition of I to the sample.

The structure of II is unknown, however, two fluorescent compounds with different mobilities toward the anode (1.6 cm and 2.8 cm) were found by paper electrophoresis (1 m HCOOH, pH 1.75, 36 v/cm, 10 min).

Extension of the present method to fluorometric determination of inorganic and organic thiosulfates (Bunte salts), polythionates etc. is now under way. A more complete account of the present procedure will be given in the near future.

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Received May 25, 1974

Evidence for O-Methylation of Catechol Estrogen 2-Glucuronoside with Retention of Conjugate in the Rat

It has previously been demonstrated that O-methylation occurred at the nonconjuncted phenolic group of catechol estrogen monoglucuronoside in the rat.1) However, whether the glucuronoside linkage would be retained intact during O-methylation or not still remains unclear. In this paper we wish to present a definite evidence for in vitro formation of 2-hydroxyestrone 3-methyl ether 2-glucuronoside with retention of the inherent sugar moiety.

An initial effort was directed to preparation of 2-hydroxyestrone 2-glucuronoside-14C as a substrate. It is substantiated that the rat, hamster, and guinea pig are capable of catalyzing the glucuronyl transfer exclusively at the C-2 phenolic group of catechol estrogen.2) Incubation of 2-hydroxyestrone with rat liver homogenate in the presence of uridine diphosphogluconic acid-14C afforded the desired 2-hydroxyestrone 2-glucuronoside-14C in a satisfactory yield.

Fresh liver from male Wistar rats (ca. 250 g) was homogenized in ice-cooled 0.25 M sucrose solution to a final concentration of 20%. The homogenate was centrifuged for 20 min at 1500 × g and the supernatant was used for the incubation study. The incubation mixture contained liver homogenate (6 ml), 2-hydroxyestrone 2-glucuronoside-14C (0.73 μCi, 8.3 mg), [3H-methyl]-S-adenosylmethionine (2.5 μCi), magnesium sulfate (4 mg), p-glucaro-1,4-lactone (1 mg), and 0.1 M phosphate buffer (pH 7.5) to make a final volume 8 ml. The mixture was incubated for 90 min at 37°. The incubation mixture was deproteinized with ten-fold volume of ethanol and the supernatant was evaporated in vacuo. To the residue was added 2-hydroxy-