Table III—Recovery Data of Dantrolene Sodium[a]

Sample	D/IS Ratio[b]	Recovery[c], %
Water	4.0368 ± 0.2148	99.4
Plasma	3.3620 ± 0.1916	82.7
Whole blood	2.7903 ± 0.0312	68.5

[a] Ten micrograms of dantrolene sodium was added to each sample. [b] Based on triplicate injections of three individual samples. [c] Calculated from linear regression analysis data.

concentrations were calculated from the constants (slope and intercept) obtained from the linear regression analysis. The concentration was calculated from the equation: D/IS = (slope × concentration) + intercept, using a programmable calculator[11]. Accuracy was 1–3% for the overall procedure.

The results for the spiked biological samples obtained from HPLC and fluorometry are shown in Table II. There was no significant difference between the values obtained by the two techniques at $p = 0.01$.

Recovery data of dantrolene sodium from water, plasma, and whole blood are shown in Table III. Extraction of the drug from spiked plasma accounted for approximately 82% of the added dantrolene. In the whole blood investigations, about 31.5% of the drug was not found in the plasma portion. Blood cells may account for about 15% binding of the dantrolene.

In summary, this procedure is useful for the determination of drug in the presence of its two major metabolites with a minimum detectability of 8 ng.

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Polynitro Aromatic Compounds in Analytical Chemistry I: Reaction with Ouabain and Digitoxin

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Abstract □ By the use of NMR spectroscopy, the highly colored reaction products formed by ouabain or digitoxin with 1,3,5-trinitrobenzene or 2,4,6-trinitroanisole in the presence of alkali (as used for the determination of these glycosides) are shown to be Meisenheimer complexes. The complexes are produced by attachment of a carbon of the butenolide ring to an aromatic carbon of the nitro compound with formation of a charge-delocalized cyclohexadienate anion.

Keyphrases □ Nitro aromatic compounds—1,3,5-trinitrobenzene and 2,4,6-trinitroanisole, NMR spectral characterization of products □ Ouabain—reaction with 1,3,5-trinitrobenzene or 2,4,6-trinitroanisole, NMR spectral characterization of products □ Digitoxin—reaction with 1,3,5-trinitrobenzene or 2,4,6-trinitroanisole, NMR spectral characterization of products □ NMR spectroscopy—characterization of products of reaction of ouabain and digitoxin with 1,3,5-trinitrobenzene or 2,4,6-trinitroanisole □ Cardiotonic agents—ouabain and digitoxin, reaction with 1,3,5-trinitrobenzene or 2,4,6-trinitroanisole, NMR spectral characterization of products

The most recent review article on the chemical estimation of ouabain and digitoxin was published in 1949 by Canbäck (1). In 1950, the same author (2) reported further results on the reaction of these cardiotonic glycosides with dinitro aromatic compounds in the presence of alkali. Canbäck attributed the intense color produced to the transfer of a proton from the butenolide ring of the glycoside to a ring carbon of the nitro compound. Later, Kimura (3) investigated the reaction between digitoxin and picric acid and proposed that the colored product resulted from the addition of the butenolide ring to one of the two unsubstituted carbons of the picric acid.

In the present work, NMR studies established that ouabain and digitoxin give Meisenheimer complexes (charge-delocalized cyclohexadienate anions) with both 1,3,5-trinitrobenzene and 2,4,6-trinitroanisole in dimethyl sulfoxide as the solvent. Comparison of the visible spectra of the products with those of materials produced in aqueous methanol (which more nearly approximates the normal assay medium) suggested that the colored components are the same in the two cases.

EXPERIMENTAL[1]

Visible Spectra—Approximately 3×10^{-5} *M* solutions of ouabain and digitoxin were used in a 1-cm cell. Excess 2,4,6-trinitroanisole and sodium hydroxide were added, and the spectra were measured against a reagent blank. The positions of the absorption maxima were: ouabain–trini-

[1] Visible spectra were obtained using a Cary model 15 UV-visible spectrophotometer. NMR spectra were run on a Jeolco MH-60-II spectrometer.

Table I—Chemical Shifts (δ) of Protons of Trinitrobenzene and Trinitroanisole in Dimethyl Sulfoxide-d_6 as Affected by Base, Ouabain, and Digitoxin

Nitro Compound	Glycoside	Base	Chemical Shifts of Lowest Field Protons	Chemical Shift of Methoxyl Protons
Trinitrobenzene	None	None	9.04	—
Trinitrobenzene	None	Sodium hydroxide	7.68, 5.61[a,b]	—
Trinitrobenzene	Ouabain	None	9.04	—
Trinitrobenzene	Ouabain	Sodium hydroxide	8.17, 6.07[a,b]	—
Trinitrobenzene	Digitoxin	None	9.07	—
Trinitrobenzene	Digitoxin	Sodium hydroxide	8.23, 6.10[a,b]	—
Trinitroanisole	None	None	9.00	4.07
Trinitroanisole	None	Sodium hydroxide	8.56[a]	3.13
Trinitroanisole	Ouabain	None	9.00	4.07
Trinitroanisole	Ouabain	Sodium hydroxide	8.56[a]	3.30[c]
Trinitroanisole	Digitoxin	None	9.00	4.07
Trinitroanisole	Digitoxin	Sodium hydroxide	8.56[a]	3.30[c]

[a] Spectra also showed aromatic protons of the unchanged nitro compound. [b] Integrated areas of these peaks were in the ratio of 2:1. [c] Area around δ 3.30 was obscured by water peak unless concentration was carefully controlled. A solvent interaction peak at δ 3.13 also was present.

troanisole–base, λ_{max} 536 (aqueous methanol) and 545 (dimethyl sulfoxide) nm; and digitoxin–trinitroanisole–base, λ_{max} 536 (aqueous methanol) and 545 (dimethyl sulfoxide) nm.

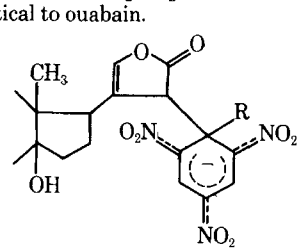
NMR Spectra—The spectra of the nitro aromatic compounds were obtained in dimethyl sulfoxide-d_6, and then ouabain or digitoxin (presumably in excess) was added until the signals of the glycoside became visible. The colored products were formed by adding a small amount of sodium hydroxide in deuterium oxide, and the spectra were determined again (Table I). A reagent blank spectrum was obtained from a mixture of all components except the glycoside.

RESULTS AND DISCUSSION

The visible absorption peak developed by both glycosides with trinitroanisole in dimethyl sulfoxide was only 9 nm displaced from its position in aqueous methanol. Therefore, the colored complex in dimethyl sulfoxide probably has the same structure as that in aqueous methanol and corresponds to the species used in the chemical assay. Therefore, the NMR studies in dimethyl sulfoxide should help to elucidate the nature of the assay reaction.

The NMR spectra clearly showed that the nitro compounds did not undergo any reaction in the absence of base. Interpretation is complicated by the observation that there was complexation between the solvent and the aromatic nitro compound in the absence of a glycoside. Thus, the singlet from 1,3,5-trinitrobenzene at δ 9.04 in dimethyl sulfoxide alone was replaced by a pair of signals at δ 7.68 and 5.61 on the addition of base. When ouabain was present, the two new signals appeared at δ 8.17 and 6.07; clearly, a different complex was formed from that provided by the solvent.

The positions of the new peaks and their 2:1 intensity ratio were consistent with their representing the two protons on sp^2 hybridized carbon and the one proton on sp^3 hybridized carbon in a Meisenheimer complex (I) (4–11). Both peaks were moved upfield because of the loss of aromaticity of the ring. The chemical shift of the one-proton peak at δ 6.07 was consistent only with attachment of the new ligand at one unsubstituted carbon of the trinitrobenzene ring. Digitoxin with trinitrobenzene gave results almost identical to ouabain.



I: R = H or OCH_3

In the reactions of the glycosides with trinitroanisole and base, only one new low field peak, at δ 8.56, was seen. This finding strongly suggests attachment of the glycoside at the methoxylated carbon of the nitro compound. Since solvent and base gave a new peak also at δ 8.56, it could be argued that only solvent was involved in the complexation. However, the position of the trinitroanisole methoxyl peak proved that complexation with the glycosides was occurring; in the absence of the glycosides, it appeared at δ 3.13. When either glycoside was present, it was found at δ 3.30.

In summary, the evidence supports the view that the colored complexes formed by ouabain or digitoxin with the trinitroaromatic compounds in the presence of base may be formulated as Structure I. The butenolide ring is shown with its double bond shifted and the carbon next to the carbonyl group as the position reactive toward the nitro aromatic ring (2, 3). The present work does not provide any additional evidence about the condition of the butenolide ring.

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