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Determination of Emodin in Feeds

Emodin from feeds was extracted into aqueous acetonitrile and partitioned into chloroform. After concentration of the chloroform extract, emodin was detected by thin-layer chromatography and quantitatively determined by high-performance liquid chromatography. Recoveries from various materials averaged from 80 to 90%.

The fungal pigment emodin (1,3,8-trihydroxy-6-methylanthraquinone) is widely distributed, occurring in the genera *Aspergillus* (Wells et al., 1975), *Cladosporium*, *Chaetomium*, *Penicillium*, and *Penicillopsis* (Shibata et al., 1964). Wells et al. (1975) and Kinosita and Shikata (1965) have reported on its toxicity. In these studies, it appeared that emodin acted synergistically with other fungal metabolites. Because of its toxicity and wide distribution, a simple method for its determination in agricultural commodities would be useful. Rai et al. (1975) developed a HPLC separation for emodin and other anthraquinones, while Danilovic and Naumovic-Stevanovic (1965) described a thin-layer method for some anthraquinones. These studies, however, did not attempt the analysis of an anthraquinone metabolite from a complex mixture. A procedure utilizing a liquid–liquid partition cleanup followed by thin-layer or liquid chromatography is described.

EXPERIMENTAL SECTION

Reagents and Equipment. Emodin was purchased from the Aldrich Chemical Co. (Milwaukee, WI); solvents were commercially supplied reagent or pesticide grades. Silica gel plates were prepared from silica gel G purchased from Sigma (St. Louis, MO). Sufficient water was added to the silica gel so that bubbles were not trapped in the slurry following shaking (about 30 g of silica gel to 70 mL of water). Layers 0.25 mm thick were spread on glass plates, allowed to air-dry, and then activated for about 2 h at 120 °C. Plates were kept in a cabinet over desiccant until used.

The liquid chromatograph was a Waters Model 6000 pump with the U6K injector (Waters Associates, Milford, MA) and a Beckman Model 153 ultraviolet absorbance detector. Columns used were 25 cm × 4.6 mm i.d. with 10-μm silica gel packing (Beckman Instruments, Fullerton, CA) and 10-μm C_{18} reversed-phase packing (Alltech Associates, Deerfield, IL).

Extraction. Fifty grams of the feed or ground grain was extracted with 250 mL of 80:20 acetonitrile–water either by blending for 5 min or by mechanical shaking for 30 min. The mixture was filtered through fluted filter paper and a measured amount of filtrate taken (e.g., 150 mL). The filtrate was partitioned twice with 100-mL portions of hexane, discarding the hexane layers. Seventy-five milliliters of water was added to 150 mL of filtrate, and the aqueous acetonitrile extract was partitioned with 2 × 50 mL portions of chloroform. The lower chloroform layers were combined and taken to dryness on a rotary evaporator. The residue was transferred to a graduated tube with 9:1 chloroform–methanol and brought to a desired volume, e.g., 0.5 or 1.0 mL. An aliquot was then taken for analysis by thin-layer or liquid chromatography. (**Caution**: good ventilation is required when using chloroform and acetonitrile.)

Chromatography. Aliquots of the extract were spotted on silica gel plates, previously described, along with standards of emodin dissolved in chloroform. Many solvent systems may be employed. The following systems were used in this work (with R_f values in parentheses): 50:50:2:1 hexane–chloroform–acetic acid–methanol (0.40), 88:12:1 chloroform–acetone–water and 100:1:1 chloroform–methanol–acetic acid (0.50). Emodin is visible to the naked eye in quantities as low as 0.2–0.5 μg as a yellowish orange spot. When exposed to ammonia vapor, the spot becomes pink and more intense. Emodin exhibited a yellowish orange fluorescence under short- and long-wave ultraviolet light. Detection is sometimes complicated by oily material in the extract, which alters the R_f value and may overload the plate capacity, but 1 or 2 ppm of emodin may usually be detected by thin-layer chromatography.

High-performance liquid chromatography (HPLC) may also be utilized in the determination of emodin, particularly for quantification. While Rai et al. (1975) used a pellicular silica with a cyclohexane–ethyl acetate gradient, microparticulate silica HPLC packings were used in this study. Solvents used were 80:20:1 methanol–water–acetic acid for reversed-phase separations, while 95:5:1 isooctane–2–propanol–acetic acid was used with silica gel columns. At the low part per million levels, monitoring at 254 or 280 nm with the sensitivity set at 0.08 absorbance unit full scale was satisfactory. On the reversed-phase system, 0.5 μg of emodin gave about 30% full-scale deflection with a retention of about 9 min, when a monitoring wavelength of 280 nm at 0.08 absorbance unit full scale was used. Detection limits by HPLC are estimated at

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Table I. Recoveries of Emodin and Standard Deviations

matrix	ppm added	% recovery	SD
corn	0.5	81.8	9.1
	1.0	86.1	5.2
	2.0	86.2	2.3
oats	0.5	85.6	12.4
	1.0	80.7	16.8
	2.0	80.1	27.6
mixed feed	0.5	102.6	7.2
	1.0	94.0	10.0

0.2–0.3 ppm of emodin. The chief limitation is not detector sensitivity but the amount of background interference present, since the cleanup procedure is minimal. If necessary, some background interference may be reduced by partitioning the sample extract in about 2 mL of chloroform or methylene chloride with an equal volume of 1 M NaOH. The aqueous alkaline fraction is then acidified with dilute HCl, and the emodin may then be back-extracted into chloroform or methylene chloride and concentrated.

RESULTS AND DISCUSSION

Recoveries of emodin were calculated by adding known amounts of emodin to nonmolded corn, oats, and a ground swine feed. After extraction, quantification was performed by using HPLC. Three to four replicates of each concentration were determined in each sample matrix. The recoveries and standard deviations are given in Table I. Recoveries were usually fairly consistent, with the exception of oats, which gave erratic results at the higher concentrations for unknown reasons.

While HPLC determination was somewhat more sensitive than thin-layer chromatography under these conditions, the latter is probably more selective. The characteristic color reactions of emodin are important for confirmation, particularly in complex samples.

Since a number of mycotoxin analytical methods based on acetonitrile extraction have been developed (Pons et al., 1973; Stoloff et al., 1971), it would seem possible to include emodin in a multi-mycotoxin screening procedure. Other hydroxyanthraquinones might be expected to behave similarly, though emodin appears to be the most widespread and is also commerically available as a standard.

Registry No. Emodin, 518-82-1.

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Photodecomposition of a Commercial Polybrominated Biphenyl Fire Retardant: High-Resolution Gas Chromatographic Analysis

The photolytic degradation of fireMaster BP-6, a commerical polybrominated biphenyl (PBB) fire retardant, was investigated by using high-resolution, isomer-specific, fused silica capillary gas chromatography. During photolysis the more highly brominated PBB congeners diminished in concentration, while those possessing three, four, or five bromines per biphenyl increased, consistent with a reductive dehalogenation pathway. Unlike the photolysis of single cogeners, a preferential loss of ortho bromines was not found. Those PBBs which have been identified as the most toxic (i.e., those containing no ortho bromines) did, nevertheless, increase approximately 4-fold, and this increase may explain the enhanced toxicity of photolyzed fireMaster BP-6.

Studies on the photolytic degradation of isomeric polyhalogenated biphenyls have revealed the following: (1) at environmentally significant wavelengths (>290 nm) polyhalogenated biphenyls undergo a stepwise reductive dehalogenation (Safe and Hutzinger, 1971; Ruzo et al., 1972), (2) polybrominated biphenyls (PBBs) are in general more reactive than the polychlorinated biphenyls (Ruzo and Zabik, 1975), and (3) ortho halogens cleave first and at a faster rate when para halogens are present on the same ring (Ruzo et al., 1975; 1976; Bunce et al., 1975).

Recent work in several laboratories indicates that PCBs and PBBs possessing one or no ortho halogens, and two para halogens, are quite potent in causing numerous biologic effects and are the most toxic polyhalogenated biphenyl congeners (producing a wasting syndrome, lymphoid involution and immunosuppression, tetratogenicity and embryotoxicity, and porphyria; Parkinson and Safe, 1981; Robertson et al., 1982; Render et al., 1982; Kawanishi et al., 1981; Marks et al., 1981; Silkworth and Grabstein, 1982). It is reasonable then to speculate that the preferential loss of ortho halogens during photolysis should lead to polyhalogenated biphenyls which are more toxic. For example, PBB congener **153** [numbering system adopted from Ballschmiter and Zell (1980)], 2,2′,4,4′,5,5′-hexabromobiphenyl, the major component of fireMaster BP-6, would be expected during photolysis to yield 2,3′,4,4′,5-

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