den Systemen A (Dichlormethan–Äthylacetat, 9:1 v/v) und B (Chloroform–Cyclohexan–Eisessig, 5:4:1, v/v/v). Damit werden die Verluste wesentlich geringer als bei den bisher angegebenen Methoden mit mehrfacher Chromatographie.

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A spray reagent for the identification of epoxides on thin layer plates

Picric acid (2,4,6-trinitrophenol) has been found to react with a number of epoxides at room temperature[1]. The reaction is specific and has been particularly useful in detecting epoxides in heated oils where the presence of other oxidation products can make detection and isolation difficult[2]. To our knowledge no satisfactory spray reagent for fatty (internal) epoxides exists. The sodium iodide reagent used by BUCHANAN AND SCHWARTZ[3] works well on terminal epoxides only. The method which relies on the formation of halo-hydrins[4] is not completely satisfactory because it lacks specificity and also because it does not give a colored derivative. The method described below eliminates some of these difficulties.

Experimental

Materials. The preparation of the epoxidized methyl esters used in this study has been described elsewhere[1]. An oil rich in the triglyceride of vernolic acid (*cis*-12,13-epoxy-*cis*-9,10-octadecenoic acid) was obtained by crystallizing *Vernonia anthelmintica* seed oil from four volumes of 20–40° petroleum ether at —10° for 18 h. When freed from the solvent, the crystallized material contained 4.34% oxirane[5] for a computed vernolic acid content of 84%, most of which is in the triglyceride form. The oil contained 2.5% unsaponifiable matter[6] and about 12% of the natural fatty acids,

among which palmitic and linoleic are predominant. The rat lipids were obtained by chloroform–methanol (2:1, v/v) extraction of the serum, liver or epididymal fat pads. The three groups of animals had been fed a commercial diet containing 5 % corn oil (control), 1.6 % and 4.8 % trivernolin for 90 days.

Thin layer chromatography. TLC was carried out using 250 μ thick, commercially available 20 × 20 cm Silica Gel G plates (Analtech, Inc., Wilmington, Dela.). The developing system was petroleum ether (20–40°)–diethyl ether–acetic acid (75:25:1, v/v/v). After development, the plate was sprayed thoroughly with 0.05 *M* picric acid in 95 % ethyl alcohol and immediately placed in a tank saturated with the vapor of a diethyl ether–95 % ethyl alcohol–acetic acid solution (80:20:1, v/v/v). Thirty minutes later the plate was removed and exposed to ammonia fumes for 1–2 min. The epoxides appeared as orange spots on a yellow background. After these spots had been marked, the plates were sprayed with chromic–sulfuric acid solution[7] and heated at 180° for 30 min.

Results

The charring step described above visualizes all of the entities present as dark spots on a white background. The orange spots are due to the formation of the hydroxy-picryl ether adduct which in the presence of a base has a reddish-orange color[1,2]. On a TLC plate, the reaction is much faster than in solution and, even though in most cases 30 min was used, 15–90 min exposure also gives satisfactory results when fatty epoxides are analyzed. No reaction seems to take place unless the picric acid sprayed plate is kept in contact with acidic ether–ethanol vapor. When high levels of carbonyl compounds are present, *e.g.* 2,4-hexadienal, mesityl oxide, 11-ketooleic acid, a yellow orange color is also obtained. This interference can be eliminated by using pyridine instead of ammonia vapor in the last step[2]. It seems certain that the rate and the extent of reaction (sensitivity) is dependent on the environment of the oxirane moiety. Work with known fatty epoxides has shown that the method is sensitive to 5 μg of material; this is true for *cis*-methyl epoxy-stearate, methyl vernolate (Fig. 1) and trivernolin. For *trans*-methyl epoxy-stearate and methyl diepoxy-stearate the lower limit of detectability is about 10 μg. Prior experience has shown that this method would also be applicable to terminal and most other types of commercial epoxides[1].

Fig. 1 shows the results obtained using known epoxides and rat lipids. Here the shaded spots are those that gave a positive picric acid reaction. The dotted circles indicate the presence of trace amounts of sample. Lane 1 represents 50 μg of the recrystallized *Vernonia* seed oil; the upper spot is the divernoyl triglyceride, the lower spot is trivernolin. Lane 2 shows 50 μg of methyl vernolate. Lane 3 contains 30 μg of serum lipids and lane 4 contains 500 μg of liver lipids obtained from rats fed the high level of trivernolin. Lanes 5–7 show about 2 mg of lipids from the epididymal lipids from rats fed 0.0, 1.6 and 4.8 % trivernolin respectively. Lane 8, in order of increasing R_F, shows 30 μg each of mono-, di- and triglyceride of palmitic acid. The monopalmitin barely moves from the origin; sterols, when present, have an R_F similar to that of dipalmitin which in turn has an R_F identical to trivernolin. Data from this figure and other similar experiments show that epoxides are present only in the epididymal fat pads of the animals fed trivernolin. The sample from the high level feeding (lane 7) shows a faint epoxide spot which has an R_F similar to trivernolin

and a larger spot which is probably the divernoyl triglyceride. Most of the orange color (epoxides) in the sample, however, is found in the spot which, in this solvent system, has an R_F similar to methyl vernolate. GLC definitely shows that this is not the methyl ester of vernolic acid. Work is now underway to determine the identity of this epoxide-containing spot.

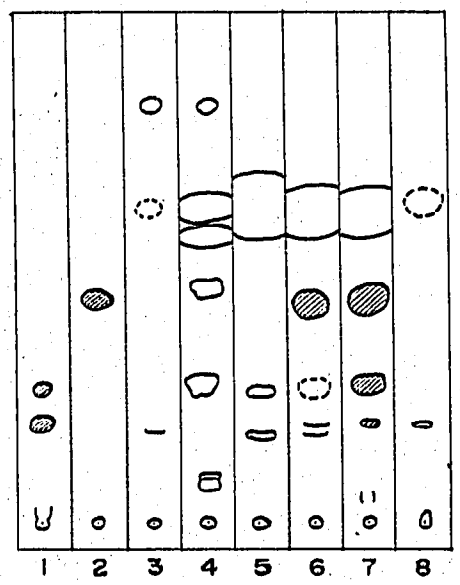

Fig. 1. TLC of rat lipids. The shaded areas represent epoxide-containing moieties.

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