betamethasone also inhibited exudate formation, body weight gain, and the thymus weights. It is difficult to obtain a relative activity of betamethasone since the concentration used was high and may represent a maximum response.

Unlike clinical observations of topical activity in dermatologic diseases (13–15) in which betamethasone 17-valerate is generally described as comparable to fluocinolone acetonide, the authors' test procedure indicates that fluocinolone acetonide is more active. An attempt to grade the order of activity from the experimental results indicates that fluocinolone acetonide (0.025%) > betamethasone (0.2%) > triamcinolone 16,17-acetonide (0.025%) > betamethasone 17-valerate (0.025%) > methylprednisolone acetate (0.25%) > hydrocortisone acetate (1.0%). This order of decreasing activity is closely related to that described by others (16, 17). Similarly, one may also correlate steroidal modifications such as the addition of Δ^1, 6-methyl, 6-fluoro, 9-fluoro, 6,9-difluoro, 16-methyl, and 16,17-acetonide to the increasing order of biological activity (5, 16, 18, 19).

In conclusion, the modified granuloma pouch procedure not only serves as a site for drug application but is also useful for the evaluation of commercially available corticosteroids. The assay can also distinguish structural modifications and alteration of the vehicle. However, these changes did not always reflect potency differences observed in the clinic.

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Gas-Liquid Chromatography of Salicylate Metabolites

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Abstract □ A gas-liquid chromatographic separation of the methyl ester-methyl ether derivatives of acetylsalicylic acid, salicylic acid, 2,3-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, and 2,3,4-trihydroxybenzoic acid is described. Separations were carried out at 155° on a column packed with 5% SE-30 on diatomaceous earth. A hydrogen-flame ionization detector was used.

Keyphrases □ Salicylate metabolites—separation, determination □ Column chromatography—separation □ GLC—analysis

It was decided to develop a gas-liquid chromatographic technique, for possible use by the authors and others, which would effect the separation and identification of acetylsalicylic acid, salicylic acid, 2,3-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, and 2,3,4-trihydroxybenzoic acid. Horning *et al.* (1) report retention times for trimethylsilylether (TMS)–methyl ester derivatives of acetylsalicylic acid, salicylic acid, gentisic acid (2,5-dihydroxybenzoic acid), and salicyluric acid, but they do not mention either 2,3-dihydroxybenzoic acid, or 2,3,5-trihydroxybenzoic acid. Williams (2) reports retention times for methyl ester and methyl ester–methyl ether derivatives of several dihydroxybenzoic acids including 2,3- and 2,5-dihydroxybenzoic acids but he presents no data for acetylsalicylic acid, salicylic acid, or 2,3,5-trihydroxybenzoic acid. The following technique will effectively separate acetylsalicylic acid from its hydroxy metabolites.

Table I—Relative Retention Times

Methyl Ester–Methyl Ether Derivative of	Relative	Retention Time
Benzoic acid (internal standard)	—	1.00[a]
Salicylic acid	1.240	2.12
Acetylsalicylic acid	—	2.91
2,5-Dihydroxybenzoic acid	—	3.37
2,3-Dihydroxybenzoic acid	—	3.91
2,3,4-Trihydroxybenzoic acid	—	8.43

[a] Retention time = 1.026 min.

MATERIALS AND METHODS

Identity of the individual compounds used was established by melting point.

An ethereal alcoholic solution of diazomethane was prepared by reacting an ethereal solution of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide with ethanolic potassium hydroxide in a distilling apparatus (3). The resulting ethereal distillate contained approximately 3 g. of diazomethane.

The methyl ester–methyl ether derivatives of the respective compounds were prepared as follows. Approximately 0.25 g. of each compound was dissolved in a minimum amount of absolute ethanol. Ethereal diazomethane was added dropwise until a yellow color

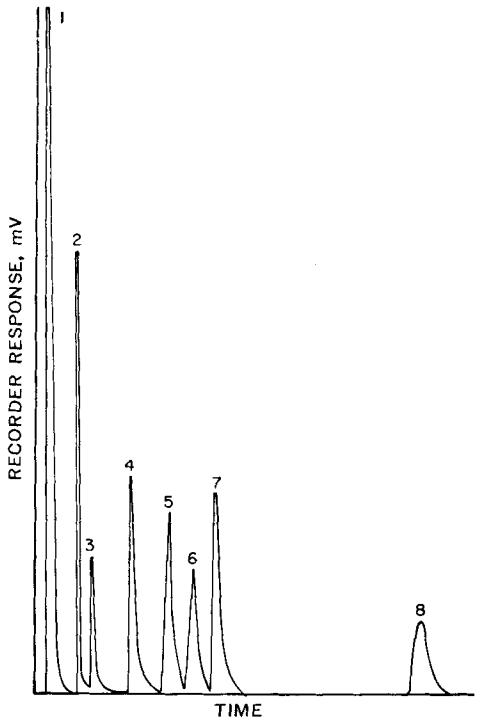

Figure 1—*Gas chromatograph showing:* (1) *solvent peak; derivatives of;* (2) *benzoic acid;* (3 *and* 4) *salicylic acid;* (5) *acetylsalicylic acid;* (6) *2,5-dihydroxybenzoic acid;* (7) *2,3-dihydroxybenzoic acid; and* (8) *2,3,4-trihydroxybenzoic acid.*

persisted, after which approximately a 20% excess by volume of ethereal diazomethane was added. The reactions were allowed to proceed at room temperature for 48 hr. in stoppered flasks. Additional ethereal diazomethane was added periodically if the resulting solutions became clear. After the reaction was completed, the solvent was evaporated at room temperature under a stream of dry nitrogen. The residual yellow viscous methyl ester–methyl ether derivatives were transferred quantitatively to 10-ml. volumetric flasks and brought to volume with pyridine.

An Aerograph 200, model 2041B (Wilkens Instrument and Research Inc., Walnut Creek, Calif.) equipped with a hydrogen-flame ionization detection system was used. Separations were carried out at 155° with a 1.524 m. (5 ft.) × 1.75 mm. i.d. stainless steel column packed with 5% SE-30 on diatomaceous earth[1] 60-80 mesh. Injector and detector temperatures were maintained at 205°. The helium carrier gas flow rate was 25 ml. per minute and the hydrogen pressure was maintained at 10 p.s.i.

RESULTS AND DISCUSSION

The relative retention times of the respective derivatives, as compared with the retention time of the methyl ester of benzoic acid, are reported in Table I. Figure 1 represents a chromatograph of a mixture of the respective compounds. Salicylic acid yielded two peaks with the same relative retention times following three separate derivative preparations. The purity of the salicylic acid was confirmed by TLC. 2,3,4-Trihydroxybenzoic acid was used as a model for the behavior of the 2,3,5-trihydroxybenzoic acid metabolite. It is not unreasonable to assume that the two compounds would demonstrate similar behavior toward the GLC system used. Since the retention time of the trihydroxy derivative is much longer than any of the other hydroxy derivatives, one would not expect the 2,3,5-trihydroxy metabolite to interfere with the analysis of the other compounds. The derivatives were found to be stable for a period of several weeks when stored at 4°.

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[1] Chromosorb W, Applied Science Laboratories Inc., State College, Pa.

New Synthesis of *rac.* Anhalonidine and *rac.* Pellotine

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Abstract □ A new synthesis of *rac.* anhalonidine and *rac.* pellotine is reported. The procedure, a modification of the method of Bobbitt *et al.* (5) for the synthesis of 1,2,3,4-tetrahydroisoquinolines, is simpler and gives better yields than those previously reported.

Keyphrases □ Anhalonidine, racemic—synthesis □ Pellotine, racemic—synthesis □ IR spectrophotometry—structure □ NMR spectroscopy—structure

The need for quantities of anhalonidine and pellotine for use in the biosynthesis of the peyote alkaloids and the inability to obtain them commercially or in sufficient quantities from the peyote cactus required that they be synthesized. Reported syntheses (1–4) are either lengthy or the yields are low. The method of Bobbitt *et al.* (5) for the synthesis of related tetrahydroisoquinolines was modified for the synthesis of these alkaloids as shown in Fig. 1.

3,4-Dimethoxygalloacetophenone (I), prepared by the method of David and Kostanecki (6), was condensed with aminoacetaldehyde diethylacetal to give Schiff's base (II) which was conveniently reduced at room temperature with sodium borohydride to yield *N*-[2-(3′,4′-dimethoxy-2′-hydroxyphenyl)ethyl]aminoacetaldehyde diethylacetal (III). Compound III was cyclized in 8 *N* HCl at room temperature to yield 4,8-dihy-