Steroid Borates III

Use in Preparation of Triamcinolone 21-Hemisuccinate and Other 21-Monoesters

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The syntheses of pure 21-hemi-acid esters of adrenocorticoids possessing the 16α,17α-dihydroxy moiety have previously not been reported. Preparation of these compounds has been dependent upon selective separation of a mixture of 21-mono- and 16,21-diesters. A method for the direct synthesis of pure 21-monoester is described employing the steroid borate complex as a reaction intermediate. This procedure is adaptable as a general synthesis for all 21-monoesters of steroids having the above-mentioned *cis*-diol configuration.

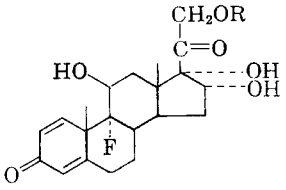
PART II of this series (1) discussed a method of preparing 21-monoesters of 16α,17α-hydroxylated adrenocorticoids. This consists of allowing the steroid to react with an acid anhydride and separating the desired 21-monoester, from the 16,21-diester, by its preferential solubility in aqueous sodium tetraborate solution.

The above procedure, although useful for simple esters, is not applicable to hemi-acid esters, such as the succinate, phthalate, or maleate (1). These latter compounds are important in pharmaceutical formulations since their sodium salts demonstrate a marked increase in water solubility over that of the parent steroid. This report describes a method of preparing such esters by reacting triamcinolone[1] and triamcinolone-like steroids, as the borate complex (2), with a dicarboxylic acid anhydride. To the authors' knowledge, this is the only direct synthesis of these compounds which does not require a selective separation step, such as a chromatographic procedure. Furthermore, this process has been found to be applicable as a general preparative method for triamcinolone 21-monoesters, represented by structure I, and is considered superior to the procedure described previously (1).

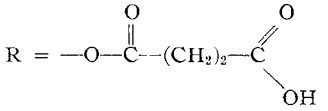
EXPERIMENTAL

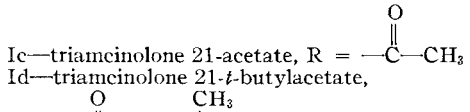
Chromatography.—A paper chromatographic system was employed for determining the nature of the products formed in the reaction between triamcinolone and succinic anhydride. The system which gives maximum resolution is butanol/0.05–0.10 *N* ammonium hydroxide using Whatman No. 1 paper. The color was developed with blue tetrazolium spray (3, 4).

Employing this system the R_f value for triamcinolone was found to be 0.8. The corresponding values for the 21-monohemisuccinate and 16,21-dihemisuccinate esters were 0.4 and 0.1, respectively.

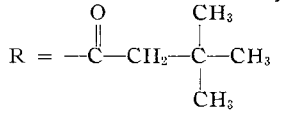


Ia—triamcinolone, R= —H
Ib—triamcinolone 21-hemisuccinate,





Ic—triamcinolone 21-acetate, R = —C(=O)—CH$_3$
Id—triamcinolone 21-*t*-butylacetate,



Investigation of Reaction Conditions for Hemisuccinate Formation.—*Temperature Study.*—Equimolar amounts of triamcinolone and succinic anhydride were dissolved in pyridine (at a 5–25% steroid concentration) and permitted to react at temperatures of 0, 25, 37, 60, and 100°. At various time intervals a sample was removed, spotted directly onto a paper strip, and chromatographed using the previously described system. The nature of the reaction products was obtained from each chromatogram.

Reactant Ratio Study.—Pyridine solutions were prepared as described above with succinic anhydride and triamcinolone in molar reaction ratios of 3:1, 5:1, and 10:1. These were stored at 100°. At various time intervals samples were removed and chromatographed as in *Temperature Study.* The identity of the products was determined from the chromatogram.

Preparation of Triamcinolone 21-Hemisuccinate (Ib).—One gram of triamcinolone sodium borate (2) was dissolved in 5 ml. of pyridine, and 0.5 Gm. of succinic anhydride added. The solution was permitted to stir overnight and poured into 100 ml. of iced, dilute (2 *N*) hydrochloric acid solution.

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The authors are indebted to Mr. Louis Brancone and coworkers for obtaining the microanalytical data and to Mr. William Fulmor and his staff for their help in interpreting the infrared and ultraviolet spectra of these steroid derivatives.

[1] American Cyanamid Company's trademark for triamcinolone is Aristocort.

The observed precipitate was collected and, when submitted to paper chromatography, showed only one spot with the R_f value of 0.4. Recrystallization from aqueous methanol resulted in a white crystalline solid in approximately 75% yield; m. p. 219–223° (uncor.); E_{239} = 16,000; $[\alpha]_D^{25}$ + 60.5° (CH_3OH). The infrared spectrum was in agreement for the proposed compound.

Anal.—Calcd. for $C_{25}H_{31}FO_9$: C, 60.72; H, 6.32; F, 3.84; neut. equiv., 494. Found: C, 60.93; H, 6.46; F, 4.16; neut. equiv., 503.

Preparation of Triamcinolone 21-Acetate (Ic).—Triamcinolone sodium borate, 1.25 Gm., was dissolved in 4 ml. of dimethylformamide and 0.2 ml. of pyridine. Acetic anhydride, 0.4 ml., was added dropwise and the reaction mixture permitted to stir for six to seven hours at room temperature. The solution was poured into 25 ml. of cold 2 *N* hydrochloric acid solution. The observed precipitate was collected and recrystallized from aqueous acetone; m.p. 215–217° (uncor.); E_{237} = 15,800; $[\alpha]_D^{25}$ + 74.5° (CH_3OH). An infrared spectrum indicated this material to be triamcinolone 21-monoacetate.

Anal.—Calcd. for $C_{22}H_{28}FO_8$: C, 63.29; H, 6.70; F, 4.35. Found: C, 63.35; H, 6.71; F, 4.57.

Preparation of Triamcinolone 21-Tertiarybutylacetate (Id).—One-half gram of triamcinolone sodium borate was dissolved in 7 ml. of pyridine, and 1 ml. of *t*-butylacetyl chloride introduced. The solution was mixed for six hours at room temperature, after which it was added to 40 ml. of water. Cold 2 *N* aqueous hydrochloric acid solution was introduced until there was no further precipitation. The solid was collected and recrystallized from acetone-petroleum ether solution resulting in a white crystalline solid; m.p. 221–222° (uncor.); E_{237} = 15,000; $[\alpha]_D^{25}$ + 74.3° (CH_3OH). The infrared spectrum indicated it to be triamcinolone 21-*t*-butylacetate.

Anal.—Calcd. for $C_{27}H_{37}FO_7$: C, 65.83; H, 7.57; F, 3.86. Found: C, 65.93; H, 8.11; F, 3.82.

DISCUSSION

It would be expected that a reaction involving equimolar quantities of triamcinolone (Ia) and succinic anhydride would result in the compound triamcinolone 21-hemisuccinate. Early experiments, however, indicated the product to be a mixture of starting material, and the 21-mono- and 16,21-dihemisuccinate. This conclusion was based on elemental analyses, neutralization equivalent data, and infrared spectra. A 2:1 molar reaction ratio of anhydride to steroid yielded similar results. It was therefore decided to investigate the effect of reaction conditions on the nature of the products to determine whether simple variations in these conditions would result in formation of only 21-monoester. The previously described paper chromatographic system was employed in this study.

Upon chromatography of material obtained from the reaction between triamcinolone and succinic anhydride, three spots were observed on the strips. The first of these (R_f = 0.8) was readily identified by means of a control, as triamcinolone. However, since authentic samples of the esters were unavailable, the identities of the two remaining spots were assigned on the basis of polarity. Therefore, because of their respective polarities, the second and third spots (R_f = 0.4 and 0.1) were identified as the 21-mono- and 16,21-dihemisuccinate esters. It was confirmed that these assignments were correct when pure monoester was prepared and characterized by physical and chemical analysis.

Investigation of Reaction Conditions.—When equimolar quantities of triamcinolone and succinic anhydride were reacted at temperatures from 0 to 100°, samples were removed over varying time periods of from < one minute to seven days. The results, as interpreted from paper chromatograms, indicated that regardless of temperature a 1:1 steroid to anhydride reaction ratio produced a mixture of starting material, monoester, and diester. As the temperature approached 100°, the major components appeared to be starting material and diester.

When succinic anhydride and triamcinolone were reacted in 3:1, 5:1, and 10:1 molar ratios for time periods of from < one minute to seven hours, two new spots were encountered on the paper chromatograms. The first of these had an R_f value of 0.35 and was considered to be the 16-monoester. The second, with the R_f value of <0.1 (approx. 0.06), was interpreted as the 16,17,21-triester. However, these materials, not being obtained in significant amounts, were felt to be of minor interest.

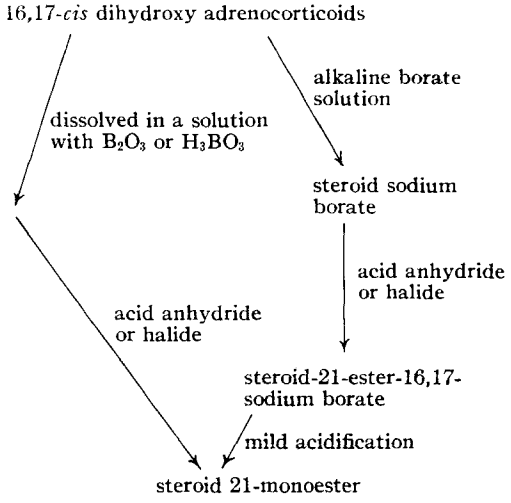
The results of these experiments, interpreted from the paper chromatograms, indicated that after a reaction time of three hours the major component was the diester. It was therefore concluded that pure triamcinolone 21-hemisuccinate could not be prepared directly by using succinic anhydride and triamcinolone regardless of the temperature or molar reaction ratios. As a result, a new procedure for preparing the monoester was investigated.

Reaction Procedure Employing the Steroid Borate Complex.—Since it was recognized that the 16-hydroxyl group of triamcinolone sodium borate was involved in complex formation (2), the possibility that this position would not be available in a reaction between the steroid borate and succinic anhydride was considered. When triamcinolone sodium borate was combined with succinic anhydride at room temperature for twenty-four to seventy-two hours in a 1:1, 1:2, or 1:3 molar ratio, a paper chromatogram of the product showed only one spot (R_f = 0.4). Elemental analyses, neutralization data, and infrared spectra demonstrated the material to be triamcinolone 21-hemisuccinate. This ester was easily purified by recrystallization. Furthermore, this synthetic procedure is readily adaptable as a production process. In addition, it was found that replacing triamcinolone sodium borate with a solution of triamcinolone and boric oxide or boric acid gave the same results.

Since this technique performed so well in the preparation of 21-hemi-acid esters, it was investigated as a general method of preparing 21-monoesters of triamcinolone and triamcinolone-like steroids. To demonstrate this, triamcinolone 21-monoacetate and 21-mono-*t*-butylacetate were prepared and characterized. The general process is summarized in scheme A.

Some additional monoesters made by using scheme A are listed as follows: triamcinolone 21-propionate and butyrate; 1,2-dihydrotriamcinolone 21-acetate,

Scheme A



propionate, butyrate, and *t*-butylacetate; triamcinolone 21-hemiphthalate and hemimaleate; and 1,2-dihydrotriamcinolone 21-hemisuccinate, hemiphthalate, and hemimaleate.

SUMMARY AND CONCLUSIONS

1. The difficulty of preparing triamcinolone 21-monohemisuccinate by reacting succinic anhydride and triamcinolone has been discussed along with a method for studying the course of this reaction via a paper chromatographic technique.

2. A process has been described for preparing triamcinolone 21-monohemisuccinate in good yield by reacting triamcinolone borate with succinic anhydride.

3. The general application of this process for preparing 21-monoesters of 16-hydroxylated adrenocorticoids is discussed.

REFERENCES

(1) Leeson, L. J., Lowery, J. A., Sieger, G. M., and Muller, S., THIS JOURNAL, **50**, 606(1961).
(2) Leeson, L. J., Lowery, J. A., Sieger, G. M., and Muller, S, *ibid.*, **50**, 193(1961).
(3) Wagner, J. G., Dale, J. K., Schlagel, C. A., Meister, P. D., and Booth, R. E., *ibid.*, **47**, 580(1958).
(4) Mader, W. J., and Buck, R. R., *Anal. Chem.*, **24**, 666(1952).

Reduction of Tetramine Toxicity by Sedatives and Anticonvulsants

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This investigation was undertaken for the purpose of obtaining information which might be applicable to the relief of accidental intoxication with tetramine, a compound which has been used as an antifungal agent and rodent repellent. Screening studies were first made on white rats using seven different compounds possessing sedative and anticonvulsant properties to determine their effectiveness in counteracting the convulsant and lethal effects of tetramine. From these qualitative studies, sodium bromide, sodium phenobarbital, and sodium barbital were found to be most effective and were chosen for further investigation. Each of these three compounds afforded protection against a lethal dose of tetramine when administered to Swiss white male mice in a small percentage of its median lethal dose.

IN A PREVIOUS publication Haskell and Voss (1) reported findings obtained from animal studies using tetramine in the form of a saturated aqueous solution. It was found that tetramine was essentially a central nervous system stimulant with greatest action on the brain stem, and that it induced clonic convulsions which were the immediate cause of death in lethal doses. Tetramine was found to be readily absorbed from mucous membranes in aqueous solution and, in saturated aqueous solution given intraperitoneally to rats, to be destroyed at a rate somewhat less than one-fourth the median lethal dose (LD_{50}) per day. The saturated aqueous solution, when stored at room temperature in nonactinic containers, was found to remain stable for periods up to five months.

Tetramine was reported by Hagen (2) to be approximately five times as toxic as strychnine for mice, the LD_{50} in this animal being 0.1 to 0.2 mg./Kg. wt., and for the albino rat, 0.22 mg./Kg.

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