SHORT COMMUNICATIONS

NUCLEOPHILIC SUBSTITUTION REACTIONS IN STEROID SERIES. PART II

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Steroidal thiols have previously been prepared by the muleophillic displacement of sulphonic esters of steroidal alcohols with thiourea [1–3] potassium thiocyanate[4,5] or potassium thioacetate[6] in suitable solvents, followed by reductive hydrolysis of the resulting intermediates.

The authors wish to report a novel method for the preparation of thiosteroids by the nucleophilic displacement of sulphonic esters of steroidal alcohols with tetra-*n*-propyl-or tetra-*n*-butyl-ammoniumthioacetate. These reagents give an encouraging yield of the thioacetate at C_3 in cholestane series.

The thioacetates were prepared by refluxing the sulphonic esters with tetra-*n*-butyl-or tetra-*n*-propyl ammonium thioacetate in dry methyl ethyl ketone for 36-48 hr. The reaction product in each case was monitored by TLC at different intervals of time. Methyl ethyl ketone was distilled off under reduced pressure and the residue extracted with ether, washed with water and sodium carbonate solution and dried (Na_2SO_4). The solvent was distilled off and the residue was crystallised from a suitable solvent. The mother liquor was separated chromatographically.

The authors have also observed that the hydrolysis of 3α-acetylthiocholestane in methanol, under the same conditions as those used by Swann and Turnbull,[6] gives only disulphide and not cholestan-3α -thiol. However, reductive hydrolysis with lithium aluminium hydride of 3α-acetylthiocholestane in ether furnished cholestan 3α -thiol in 80% yield.

Reaction of tetra-*n*-propyl or tetra-*n*-butyl ammonium thioacetate with steroid sulphonate gives some water-soluble material which prevents complete recovery of the reaction product with ether. The water-soluble product might be some quaternary ammonium salt formed by the reaction of trialkylamine with steroid sulphonate.

Tetra-*n*-propyl or tetra-*n*-butyl ammonium thioacetate was prepared by neutralising the respective ammonium hydroxide (tetra-*n*-propyl or tetra-*n*-butyl ammonium hydroxide) with thioacetic acid at room temperature and concentrating at 60-70° to a thick syrup. The syrup was dried in a desiccator over phosphorus pentoxide. It formed dirty white crystals.

TABLE.—REACTIONS OF STEROIDAL SULPHONIC ESTERS WITH TETRA-*N*-ALKYL THIOACETATE IN METHYL ETHYL KETONE.

	Sulphonic ester	Reagent	Olefines (%)	Products	
				3-SAc%	3-01%
1.	3β-Tosyloxy-5α cholestane	Tetra-*n*-butyl ammonium thioacetate	12.2	64.2(3α-) m.p. 120°	4.2(3α-) m.p. 182°
2.	3α- Mesyloxy-5α-cholestane	Tetra-*n*-propyl ammonium thioacetate	20	54.0(β-) m.p. 109°C	3.0(3β-) m.p. 142°C
3.	3β-Tosyloxy-cholest-5-ene	do	12 hexane ν_{234} (ε,12000)	24.7(3β-) m.p. 120°C	19.9(3 β-) m.p.149-50°C
4.	Methyl 7α,12α-dihydroxy-3α-tosyloxycholanate, m.p. 74-76°C[a]	do	—	36.7(3β-) Methyl-7α-12α-dihydroxy-3 β-acetyl mercapto-cholanate m.p. 170°	Unidentified

a m.p. of foam, TLC showed one spot.

Further work on the possibilities of synthesising thiol derivatives with these reagents at different positions of steroid nucleus and identification of water-soluble product is under progress.

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ACETATE PEELS FOR THE STUDY OF CARBONATE ROCKS

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The use of cellulose peels in carbonate petrography was suggested by Appel.[1] The peels were made by applying cellulose nitrate solution to etched rock surfaces. This technique did not draw much attention from sedimentary petrologists because of the following disadvantages: the thickness of the peel was uncontrollable, the peel usually required 24 hr to dry, and it was difficult to remove from the rock surface.

More recently, commercial acetate film has been used instead of cellulose nitrate solution.[2] The use of this film has provided a much more rapid method of preparing peels than by using the nitrate solution.

The technique described in this note provides a rapid method of preparing acetate peels of carbonate rocks with an excellent textural image and practically free of shrinkage and curling. The technique involves the transfer of an image of an etched surface to a thin acetate film. The etched surface is flooded with acetone. The acetate film is then rolled on the surface without pressing with fingers, and is allowed to dry for 2 hr. When completely dry, it is peeled from the surface, and yields a remarkably detailed image of the etched surface.

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Technique

The following technique, presented in outline form, has been found very effective for carbonate rocks, and is offered here as a method which can be utilized for other rock types. It is a modification of the technique suggested by McCrone.[2]

Equipment.—Diamond saw, grinding laps and grinding powders (220-grit and 600-grit), dilute hydrochloric acid (10%), acetone, acetate film (0.003 in. thick), Duco cement, plastic tape, slide glass (3-1/4 in.×4-1/4 in.), modeling clay, and Alizarine Red-S.

Preparation of the Surface to be Peeled.—Cut a flat surface on the specimen. Grind this surface with 200-grit carborundum powder, until all saw marks disappear. Polish the surface with 600-grit powder. Wash and dry the specimen thoroughly. Etch the polished surface for a few seconds, depending on the nature of the rock, in dilute hydrochloric acid (10%). Recommended etching times are: pure dolomite 40 sec, dolomitic limestone 30 sec, and pure limestone 15 sec. Rinse the etched surface in water to remove the acid, and dry the specimen.

Making the Peel.—Fix the specimen in a lump of modeling clay so that the etched surface is horizontal. Cut a piece of acetate film, slightly larger than the etched surface. Hold opposite edges of the film and bend it downwards into a U-shape. Flood the etched surface with acetone. Immediately touch the *base* of the U to the surface and progressively roll the film onto the surface of the rock. Allow the specimen to dry completely, usually for 2 hr, to minimize curling and shrinkage. Remove the peel by grasping a free edge and pulling gently. If it catches on vugs, free with a razor blade. The peel should be stored flat.

Making Stained Peels.—In order to enhance the contrast of the minerals in the peel, it is possible to use various staining techniques and to record the coloured portions in the peel. Bissell[3] and Katz and Friedman[4] have suggested a technique by which peels may be combined with stains. This is achieved by transferring the layer of stain from the rock surface to an acetate peel.

The following method has been found to be most suitable: Prepare a solution of Alizarine Red-S, by dissolving 1 g of this dye in 1000 ml of 0.2% hydrochloric acid. Apply this dye to the etched surface until all the surface is covered with a thin layer of the solution. Leave the specimen for

exactly 5 min. Wash the specimen with water; avoid directing a stream of water onto the stained surface. Dry the stained surface and flood it with acetone. Apply the acetate film as described in a preceding paragraph. Imprints of calcite on the acetate film will appear as deep red; dolomite will remain colourless.

Mounting the Peel.—Having obtained a satisfactory peel, mounting for study can be done by either of two ways. If it is desired to study the specimen in gross detail, the peel can be mounted between two glass slides by the following method: Clean two glass lantern slides (3 1/4 in. × 4 1/4in.). Place the peel between the two slides. Trim off the excess peel and press the two slides tightly together. Bind the slide with plastic tape and label it.

Peels prepared in this manner are best studied if used in a lantern-slide projector. If it is desired to study peels under the polarizing microscope, they can be mounted in Duco cement in the same way in which a thin section is mounted in Canada balsam.

Conclusions

The technique discussed in this note is very simple, and with a little practice one can obtain optimum results. The rock peel could largely replace the thin section in carbonate petrography. In fact, the peel offers certain advantages over the thin section: greater area, ease of preparation, and possibility for use as a slide for projection. The peels can also be used like photographic negatives to make enlarged photographs. A print made from the peel of a skeletal calcarenite is shown in Fig. 1. In summary, peels provide exact reproductions of etched surfaces and can be used to evaluate texture, fabric, structure, and mineralogy of carbonate rocks.

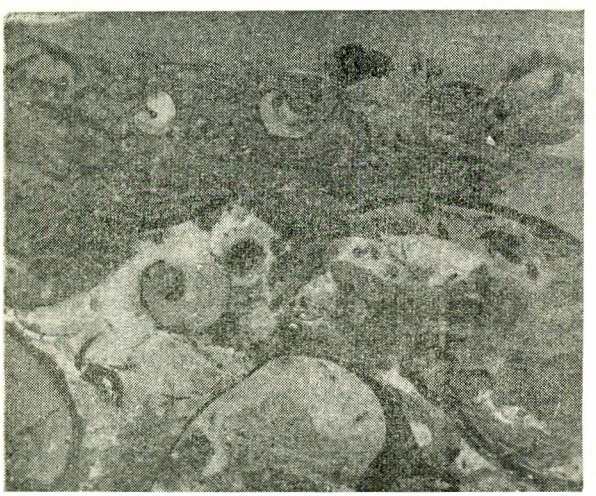

Fig. 1.—Nagative print of an acetate peel of skeletal calcarenite

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A RAPID METHOD FOR THE ESTIMATION OF THIAMINE IN MEAT

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Thiamine is estimated by measuring the intense blue-violet fluorescence of its oxidation product thiochrome.[1] A base exchange zeolite (Decalso) has been used[2–4] to separate thiamine from the interfering substances. The elution, however, takes considerable time. In the estimation of thiamine from yeast, wheat and germs by the thiochrome method, MeC Farlane and Chapman[5] have destroyed the interfering pigment by the addition of hydrogen peroxide before extraction with isobutanol.

In the case of meat the authors have found that no pigment interferes in the extraction of thiamine. A rapid method for estimating thiamine in meat and meat products has been evolved. This method takes 4 hr to complete the extraction of thiamine as against 24 hr required by the MeC Farlane and Chapman method. Moreover, it is simpler and does not involve the use of any costly equipment.

Experimental

Reagents and Apparatus.—Buffer solution: 68 g sodium acetate and 54.4 ml glacial acetic acid were mixed and diluted with water to 1000 ml pH 4.5.

Alkaline potassium ferricyanide: 6.0 ml 1% potassium ferricyanide solution and 10N sodium hydroxide were mixed and made up to 100 ml. This solution must be freshly prepared.

Sodium fluorescein: 10 mg sodium fluorescein was dissolved in 1000 ml water, concentration 10 μg/ml.

Thiamine solution: 0.01 g thiamine hydrochloride was dissolved in 100 ml water and, the solution kept in refrigerator. One ml of this thiamine solution was diluted, when required, to 100 ml, concentration 1 μg/ml.

Evans electroselenium fluorimeter.

Method.—Ten to fifteen g minced sample and 50 ml 0.3% aqueous papain solution were thoroughly blended in a 400-ml beaker and placed for ½ hr in a water bath 65°C. Then 50 ml N/20 H_2SO_4 was added and the contents heated for ½ hr on boiling water bath. 10 ml 2% solution of takadiastase made in acetate buffer of pH 4.5 was added and the mixture incubated at 37°C for 2–3 hr. The mixture was then centrifuged and the supernatant was transferred to a 200-ml volumetric flask. The residue was washed and the washing separated by centrifugation. The combined extract was made upto 200-ml mark. A portion of the above solution was filtered through a filter paper for the thiochrome test. 5 ml of the extract was taken in each of the Quickfit tubes for blank, test and recovery. 1 ml of 1 μg/ml thiamine solution was added to the recovery tube, and 4 ml 10N NaOH was added to the blank. To each of the test and recovery tubes, 4 ml alkaline potassium ferricyanide was added. The tubes were shaken and left to stand for 30 sec. The addition of the ferricyanide and the alkali solutions require a little practice for obtaining comparable results. 10 ml fluorescence-free isobutanol is added to each of the tubes and the tubes shaken vigorously for 1 min. By means of a separating funnel, the isobutanol layer was transferred into dry test tubes separately, each containing a small amount of anhydrous sodium sulphate for the removal of any turbidity. 6 ml of each of the three isobutanol extracts were taken into three cuvettes for fluorometry and readings taken.

$$\text{Amount of thiamine/100 g sample} = \frac{\text{test reading–blank reading}}{\text{recovery reading–test reading}} \times \frac{\text{total dilution in ml} \times \mu\text{g/thiamine addition} \times 100}{\text{ml aliquot taken} \times \text{wt of sample taken}}$$

Results and Discussion

The extraction of vitamin B_1 by the simple addition of an acid gave lower results, but when the sample was digested with pepsin in HCl at pH 2 it gave higher results. This was observed by Chapman *et al.*[5] also. The results in Table 1 show that takadiastase helps in the extraction of thiamine from meat and glands. The papain digestion as recommended by Emmelt *et al.*[6] gives low results, but the papain digestion followed by heating the sample in boiling water bath with N/20 H_2SO_4 and incubation with takadiastase solution gives maximum extraction of thiamine from meat and glands. The peptic digestion described by MeC Farlane and Chapman[5] gave the same results as that of papain digestion described in the present method. The release of vitamin B_1 was, however, quicker in the present method.

TABLE 1.—THIAMINE EXTRACTION TECHNIQUE AND THE AMOUNT DETERMINED.

No.	Digestion			No. of determinations	Meat glands			Extraction* %
	1st stage	2nd stage	3rd stage		Beef flesh μg/100 g	Kidney μg/100 g	Heart μg/100 g	
1.	N/20 H_2SO_4 at pH 2	—	10 ml 2% Takadiastase at pH 4.5	2	55	240	230	75
2.	N/20 H_2SO_4 at pH2	—	10 ml 2% Takadiastase and papain at pH 4.5	2	60	250	260	83
3.	Pepsin + HCl at pH2 incubation 24 hr	—	10 ml 2% Takadiastase at pH 4.5	4	75	290	300	--
4.	Papain + HCl at pH2, incubation 24 hr	—	do	2	65	260	250	85
5.	Papain for ½ hr at 65°C.	N/20 H_2SO_4 at pH 3, ½ hr (boiling water bath)	do	4	76	300	305	—
6.	Papain for ½ hr at 65°C.	do	—	2	70	270	280	93

*Calculated by comparing with the methods of extraction Nos. 3 and 5 as 100% extraction.

The results of analysis of meat for thiamine by our method and by the known procedure using Decalso[4] showed that there was no difference in the results. The mean of four determinations on a sample of beef by the two methods was the same, i.e. 70 μg/100 g.

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ICHTHIDION HALDEMAN, 1843 (INSECTA, COLEOPTERA, ANTHICIDAE, EURYGENIINAE): PROPOSED SUPPRESSION UNDER THE PLENARY POWERS IN FAVOUR OF RETOCOMUS CASEY, 1895

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Haldemen[6] published *Ichthidion murinum* using both the generic and specific names for the first time, but without describing the characters of the genus. The type species is *I. murinum* by monotypy. The species has been subsequently placed in *Eurygenius* Ferte-Senectere by LeConte[7,8] and Leng.[9] The generic name, *Eurygenius*, is commonly considered to have been proposed in 1848, but I have elsewhere given my reasons to believe that the publication did not appear before 1849.[3]

In 1895 Casey proposed a new generic name, *Retocomus*, to receive *Retocomus murinus* (Haldeman) and other North American species, formerly placed in *Eurygenius*.[4] If the generic names *Stereopalpus* Ferte-Senectere, 1849, and *Mastoremus* Casey, 1895, are to be considered valid names and the genera are distinct from *Eurygenius*, the only logical course is to accept the genus *Retocomus* as defined by Casey[4] and myself.[1,3] It should also be noted that the genus *Pergetus* Casey, 1895, is more similar to *Stereopalpus* than to either *Retecomus* or *Eurygenius*.[1,2]

In view of the fact that *Ichthidion* has remained unused as a senior synonym for more than hundred years it is to be considered a forgotten name or *nomen oblitum* in addition to being a *nomen nudum*.[3] In my opinion the stability and universality of zoological nomenclature is better served by the following action to be taken by the International Commission at my request:

(1) The use of its plenary powers to suppress the generic name *Ichthidion* Haldeman, 1843, for the purposes of the Law of Priority, but not for those of the Law of Homonymy.

(2) Placing the following generic name on the appropriate Official List of Generic Names in Zoology: *Retocomus* Casey, 1895.

(3) Placing the following generic name on the Official Index of Rejected Names in Zoology: *Ichthidion* Haldeman, 1843.

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