

chloride for 15–20 min. The resulting pale yellow complexes were cooled (0 to 5°C) and zinc powder (5-fold excess) suspended in absolute ethanol was added and the mixture heated on a water-bath for about 15 min. The ethanol was then evaporated off, water added and filtered. It was extracted twice with ethylacetate to remove neutral impurities, basified with ammonia and reextracted with ethylacetate. The organic layer was dried (Na_2SO_4) and evaporated to afford the corresponding amine which appeared as a slower moving single spot on TLC. The isolated yields were between 70–85% (Table I).

Similarly, secondary amides (7) and tertiary amides (10) afforded corresponding amines (9) and (12) respectively in good yields, as shown in Tables II and III.

Since these reactions involve the preparation of Vilsmeier complexes, their general applicability to other amides is expected. The above procedure bears distinct advantages to various other reductive methods^{2–12}, in selectivity of reaction, applicability and yields. Sodium borohydride would have reduced the aldehyde or keto

groups if present in the compound, but the above procedure may be found to yield selectively reduced amides without reducing other functional groups: such a study is underway.

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Chromosome counting on 79-year-old dried seaweed, *Porphyra leucosticta* (Rhodophyta)

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Summary. An adaptation of the aceto-iron-haematoxylin-chloral hydrate staining solution is described for chromosome counting of dried seaweeds. The chromosome number of *Porphyra leucosticta* (Rhodophyta) collected recently and fixed in ethanol-acetic acid compares well with material of the same species collected and dried in 1897. The significance of this new source of data, allowing the study of the old type material, is stressed in the paper.

We have been using with success the aceto-iron-haematoxylin-chloral hydrate solution³ for chromosome counting of dried herbarium specimens of *Porphyra* (Rhodophyta). A stock solution of this stain was prepared adding 4 g of haematoxylin and 1 g of iron alum ($\text{Fe NH}_4 (\text{SO}_4)_2 \cdot 12 \text{H}_2\text{O}$) to 100 ml of 45% acetic acid. Before using, 2 g of chloral hydrate were added to 5 ml of the stock solution.

A piece of fertile material previously soaked in distilled water for usually 5–15 min, depending on the characteristics of the thallus, is transferred to a solution of ethanol-acetic 1:2 for 10–30 min. Afterwards the material is put on a slide and quickly wiped with blotting paper, before adding 1–2 drops of the staining solution. The material is then heated on a spirit flame till the stain starts changing colour at the edge of the preparation, after which a coverslip is added and the material squashed.

The method described works equally well on material pressed alive or previously fixed in formalin, giving

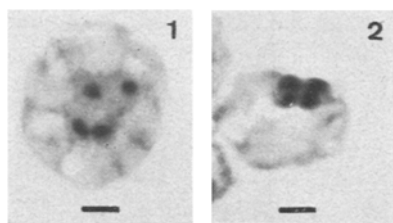
similar results when compared with fresh material fixed in ethanol-acetic acid mixtures following the usual technique.

Good preparations for observing nucleoli and chromosome counting were obtained with material dried recently or many years ago and stored under usual herbarium conditions.

To illustrate this paper, we selected pictures taken from recent gatherings of *Porphyra leucosticta* Thuret, from Uruguay, fixed in ethanol-acetic acid 1:2 (Figure 1), and of material of the same species from Phyc. Bor. Am. No. 376, collected in 1897 at Bridgeport Harbor, Connecticut, USA⁴ (Figure 2), and so almost 80 years old. The chromosome number is 4 in both samples studied.

The advantages of this method are manifold since the study of old herbarium types will certainly improve the taxonomy of this difficult and taxonomically misunderstood genus. At the moment we are engaged in the separation of several taxa in groups of equal chromosome number to which we shall apply the traditional morphological characters for species circumscription, aiming at a better understanding of the species relationship.

Although we have not yet tried this method on other genera, we believe it will work equally well once the nucleoproteins are not destroyed during the drying process.



Porphyra leucosticta. 1 Fresh material fixed in ethanol-acetic acid, showing 4 chromosomes in a spermatangia mother cell. 2 same in a material dried in 1897. Bar is 5 μm .

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