eighteen carbon atom monounsaturated fatty acid methyl esters having unsaturation in the 6-7 to 13-14 carbon atom region. Figure 1-II illustrates a spectrum of methyl petroselinate to which has been added the pseudo contact shift reagent, $Eu(fod)_3$. It is obvious that considerably more information is readily available. First to be noted is the shift downfield of a triplet (A) representing the methylene protons alpha to the carbonyl group. Next to be considered are (B) and (C), the quintets resulting from methylene protons split by methylene protons on either side. Since (D) is a quartet which results from splitting owning to both methylene and methine protons, it is therefore adjacent to an olefinic group; thus the unsaturation (E) is reasoned to be in the 6-7 position. This is confirmed by (F), a second quartet as a result of the methylene and methine splitting of the methylene protons next in the carbon chain.

The technique just described would seem to be ideally suited to locating double bond positions in monounsaturated acids in which the double bond is positioned beyond the 5-6 position. Other techniques besides the use of shift reagents can be used when the unsaturation is nearer the carbonyl group (4,5). However the effectiveness of the shift reagent diminishes as the quantity of $Eu(fod)_3$ is increased.

The technique just described was also used with varying degrees of success on methyl oleate, 11-eicosenol and oleic acid. The resolution obtained for methyl oleate at the high molar ratios of reagent was not sufficient in order to position the olefinic group in the chain. Spectra obtained for 11-eicosenol using $Eu(fod)_3$ still showed no improvement in the resolution of the methylene protons beyond the eighth carbon atom. Contrary to what had been expected, the shifts observed in the oleic acid spectrum were less than those of the corresponding ester for a given molar ratio of the reagent. This was perhaps owing to the presence of competing OH and C=O groups or a break down of the reagent. The effectiveness of the chemical shift reagent is illustrated in Figure 2 in which the chemical shift of the methylene group proton alpha to the carbonyl is plotted against the molar ratio of the europium complex to sample. Maximum effectiveness of the complex is obtained up to about a molar ration of 3:1 thereafter decreasing with increasing amounts of the complex. The $Eu(fod)_3$ was found to be completely soluble in the methyl ester. The viscosity of the mixture was great enough to require dilution with carbon tetrachloride. The shift obtained was determined to be dependent only on the molar ratio of the complex to the sample and relatively independent of the quantity of CCl_4 used, which affected only the signal strength. Another more serious problem encountered as a result of high molar ratios of the complex was a downfield shift of the $Eu(fod)_3$ proton signal which tended to mask the methylene signals at about 75 Hz as shown by the dotted line in Figure 1-II. It would also appear that the "sphere of influence" of the europium complex is an important factor. This is illustrated in Figure 2 in which it is noted that the chemical shifts of those protons on the C-atoms farthest removed from the carbonyl group are the smallest and are least affected by increases in the concentration of $Eu(fod)_3$.

Although it had been reported that shift reagents do not normally affect olefinic groups (3), a shift was observed from 315 to 352 Hz for methyl petroselinate. Since the methylene protons along the carbon chain were observed to be shifted in order of their proximity to the carbonyl group, the proton on the olefinic group closest to the carbonyl would therefore be influenced first, causing an increase in the magnitude of the downfield shift of the C_6 proton, thus making the olefinic protons nonequivalent and changing their splitting pattern. A further increase in the molar ratio shifts the protons enough to give a complex symmetrical splitting pattern of 10-12 lines which could not be analyzed as first order splitting. The proton shifted further downfield is that of the C_6 atom and that shifted least is on the C_7 atom. The implication is that substituents added to an olefinic group could thus be located positionally on the chain under optimum concentration conditions.

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A New Colorimetric Method for Estimation of Argemone Oil

ABSTRACT

Argemone oil is toxic even in low concentrations for human consumption. The author suggests a new colorimetric method in which an orange color is developed when antimony trichloride solution is added to the extracted alkaloid. The developed color is measured in a colorimeter and compared against known standards of the alkaloid. The alkaloid content of argemone oil has been found to be about 1.0%. This method determines an admixture of argemone oil as low as 0.005% in other edible oils.

Argemone oil, a common adulterant of mustard oil in India, causes serious physiological consequences to the consumer such as nausea, vomiting and headache as acute symptoms, and glocuma, edema, etc., by prolonged consumption. Even a low concentration, i.e., 0.01% of argemone oil in edible oil, is marginally safe when the edible oil is consumed in normal amounts (National Institute of Nutrition, Hyderabad, India, unpublished experiment). The seeds of Argemone mexicana resemble mustard seeds in outward appearance. Further these have a high oil content (30.0%). The alkaloids of argemone oil are known to be responsible for the toxicity of the oil. Therefore their detection in other vegetable oils and in oil cakes for animal feed is very important.

It was observed by the author that both the alkaloids of

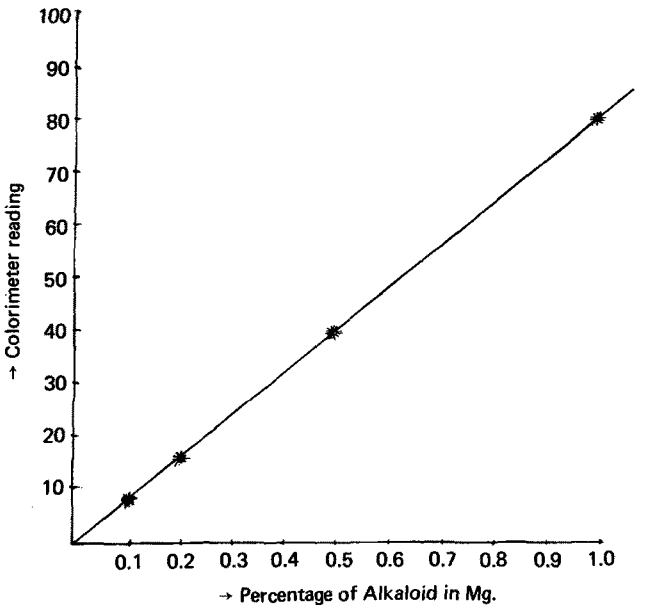

FIG. 1. Working curve for determining the alkaloid present.

argemone oil (sanguinarine and dihydrosanguinarine) after development on a thin layer chromatography plate show rapid change of color (1) to orange when sprayed with antimony trichloride solution in chloroform. In the present test, based on this color development, the quantitative determination of the toxic alkaloids is suggested. The test is as follows: 10 ml concentrated hydrochloric acid (A.R.) is added to a known quantity of oil in petroleum ether solution depending on the concentration of argemone oil present (to be ascertained by preliminary experiments) taken in a separatory funnel and shaken thoroughly. The acid layer is transferred to another separatory funnel after addition of 10 ml distilled water. The hydrochloride salts of the alkaloid are water soluble and recovered quantitatively by three additional extractions with 10 ml distilled water containing 1 or 2 drops of concentrated hydrochloric acid. The total acid extract is neutralized with 1:1 sodium hydroxide solution, cooled and shaken successively with 10, 10 and 5 ml portions of chloroform. The combined chloroform extracts are evaporated in a small beaker and kept in an air oven at 100 C to remove all moisture. The dried residue is transferred completely to a colorimeter tube with 3 ml dry chloroform. To this solution is added 2 ml 30% antimony trichloride solution in dry chloroform. The orange color is quickly measured in Klett-Summerson photoelectric colorimeter using a green filter. The solution may require dilution if the concentration is high.

A standard solution of the alkaloid is prepared by extraction from pure argemone oil and finally purified by column chromatography using silica gel as adsorbent and chloroform as developing solvent. The major alkaloid (dihydrosanguinarine constituting about 87% of the total alkaloid) is supposed to remain adsorbed on the top of the column while the other alkaloid (sanguinarine) is eluted with chloroform. Several volumes of chloroform are then passed through the column before elution of the major alkaloid with ethanol containing ammonia (90 ml ethanol + 10 ml 20% v/v ammonia). The solution containing the alkaloid is evaporated to dryness and used to prepare standard solutions at different dilutions.

Antimony trichloride solution (2 ml) is added to a known concentration of the alkaloid in 3 ml dry chloroform. The reading in the colorimeter for each sample is noted very quickly to avoid turbidity of the solution. Two precautions are taken: (a) addition of 1-2 drops of acetic anhydride in the tube before color development and (b) addition of anhydrous sodium sulphate to the antimony trichloride solution. Turbidity after color development can be removed if necessary by centrifuging. The rectilinear plot (Fig. 1) is used for calculation of the amount of alkaloid in samples.

A few samples of pure solvent extracted argemone oil were found to contain on the average about 1.0% alkaloid. Known amounts of argemone oil added in mustard oil were tested for recovery which has been found to be satisfactory. The quantity of the alkaloid estimated can be used to calculate the approximate percentage of argemone oil present in a sample of edible oil.

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