

# Application of Gas-Liquid Chromatography–Mass Spectrometry to the Analysis of Natural Products

## *Sesbania Grandiflora* (L.) Extracts

S. S. Khanna<sup>1,2</sup> and E. G. Perkins

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The ethanolic extract from the flowers of the tropical plant *Sesbania grandiflora* was examined by the direct application of gas-liquid chromatography–

mass spectrometry. A homologous series of long chain hydrocarbons (C<sub>23</sub>H<sub>48</sub> to C<sub>31</sub>H<sub>64</sub>) was identified as was an alcohol, *n*-nonacosanol.

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Use of gas liquid chromatography in conjunction with mass spectrometry (GLC-MS) has found wide applications in the field of organic chemical analysis. In the area of higher molecular weight materials, methyl esters of fatty acids (Ryhage, 1964) as well as many steroids (Eneroth, 1964) have been subjected to this technique, and Ryhage has previously demonstrated its use in identification of the hydrocarbon components of paraffin wax. The more classical techniques such as distillation and crystallization have generally been employed to separate and identify many naturally occurring wax components (Warth, 1956), and the application of combined gas chromatography–mass spectrometry to the direct identification of natural products such as long chain alcohols and waxes which occur in nature has not been widely employed. In the present study the hydrocarbons and a long chain alcohol which occurs as a portion of the lipid extracted from the flowers of the tropical plant *Sesbania grandiflora* (L.) were separated and identified using the combined GLC-MS technique.

### EXPERIMENTAL

Flowers of the tropical plant *Sesbania grandiflora* were dried at 25° C., powdered, and extracted by heating with a large volume of 95% ethanol under reflux for 8 hours. Upon filtration and cooling, a white material separated (0.03%) which was collected. The material had a melting point of 57° C.

Trimethyl silyl ether derivatives were prepared by heating 2 mg. of sample with 0.5 ml. of Bis(-tri methyl silyl) acetamide for two hours at 165° C. The silylating reagent was removed under vacuum, and the product dissolved in CS<sub>2</sub> prior to analysis.

The acetate derivative was prepared by treating 1 mg. of

sample with an excess of acetic anhydride in the presence of pyridine at room temperature for 24 hours, and the product removed by extraction with diethyl ether (Shriner, 1948).

An Aerograph model 1200, instrument with a flame ionization detector was employed. The instrument was connected via a short (10-inch heated) length of 0.01 inch S.S. capillary tubing to the heated inlet system of a Perkin Elmer Hitachi RMU-6E single focusing mass spectrometer equipped with a molecular separator of the Watson-Biemann type (1965) employed to preferentially removed carrier gas.

Gas-liquid chromatography was accomplished using a 2-foot × 1/8-inch S.S. column packed with 5% SE-30 coated on Chromasorb W., which had been conditioned for 48 hours at 350° C. Temperature programming was carried out from 150° C. to 350° C. at the rate of 8° per minute. Approximately 85–90% of each peak was diverted to the mass spectrometer for analysis as it emerged from the column; the remainder of the effluent was directed into the flame ionization detector to obtain a gas chromatographic record. A simultaneous record of the separation was also obtained by using a total ionization monitor attached to the mass spectrometer. Mass spectra of the emerging components (1–10 μg.) were recorded, near the apex of the peak, at 15-second intervals using an ionizing voltage of 20 and 70 eV. The temperature of the molecular separator and heated transfer lines was maintained at 300° C.

### RESULTS

Infrared spectroscopy of the material isolated by alcoholic extraction of *Sesbania grandiflora* flowers, indicated that it contained hydrocarbons as well as a long chain alcohol. Thin-layer chromatographic analysis of the extract employing silica gel G, according to the method of Stahl (1965) using a solvent system composed of 75:2:5 (v./v.) hexane, diethyl ether, and methanol, respectively, indicated that two major components were present. These data indicated that a direct analysis of the extracted material by combined GLC-MS, might yield an identification of the components of the mixture, since only a few milligrams were available for study. Accordingly the material was subjected to GLC-MS analysis using a

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Department of Food Science, The Burnside Research Laboratory, University of Illinois, Urbana, Ill.

<sup>1</sup> Agency for International Development Participant, 1967–68.

<sup>2</sup> Present address, Department of Physical Sciences, School of Basic Sciences, U. P. Agricultural University, Pantnagar, Dist. Nainital, U. P. India.

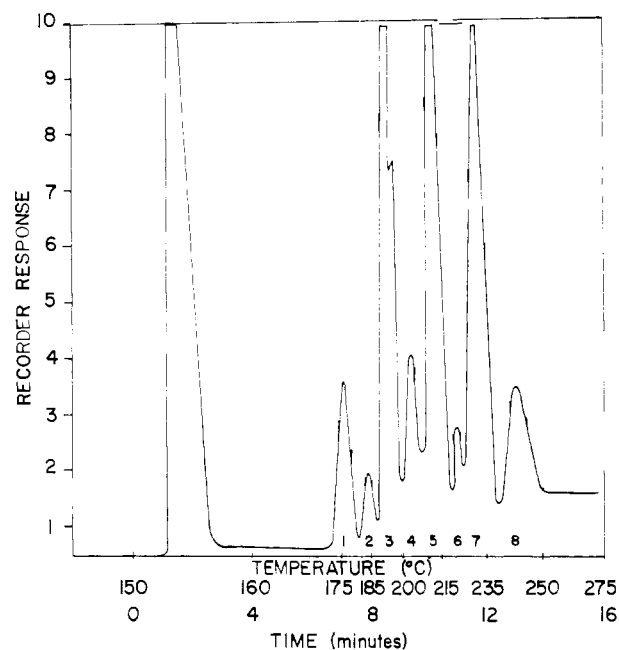


Figure 1. Total ion monitor chromatogram of *S. grandiflora* extract

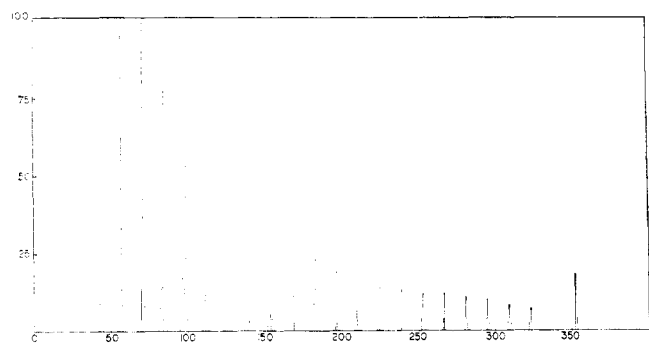


Figure 2. Mass spectrum (20 eV) of *n*-pentacosane, obtained from *S. grandiflora* extract

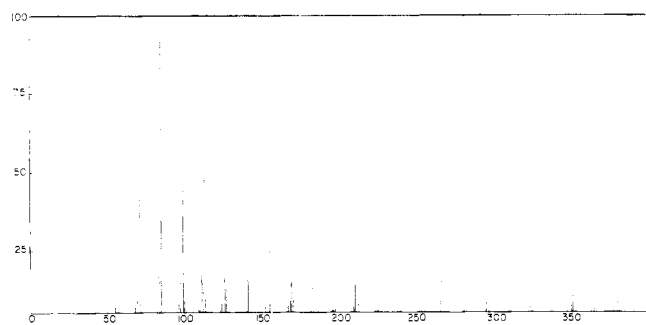


Figure 3. Mass spectra (20 eV) of nonacosanol obtained from *S. grandiflora* extract

short SE-30 column. The chromatogram illustrated in Figure 1 was obtained from the total ion monitor of the mass spectrometer. Eight peaks were obtained at a temperature range of 175° to 250° C., of which three were major components as indicated in Table I.

The mass spectrum of each peak was recorded. A typical spectrum obtained from peak number 3 (32.9%) (Figure 2 and Table I) was identified as that of the saturated hydrocarbon *n*-pentacosane. Peaks 1 to 6 and peak 8 were found to be a series of hydrocarbons by comparison of their mass

Table I. Composition of *S. grandiflora* Extract<sup>a</sup>

Peak No.	% Wt.	Molecular Formula
1	4.4	C <sub>23</sub> H <sub>48</sub>
2	1.7	C <sub>24</sub> H <sub>50</sub>
3	32.9	C <sub>25</sub> H <sub>52</sub>
4	3.2	C <sub>26</sub> H <sub>54</sub>
5	27.3	C <sub>27</sub> H <sub>56</sub>
6	3.1	C <sub>18</sub> H <sub>38</sub>
7	22.6	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>27</sub> CH <sub>2</sub> OH
8	4.7	C <sub>31</sub> H <sub>64</sub>

<sup>a</sup> Determined by gas-liquid chromatography.

spectra with those of various known standard hydrocarbons. Since examination of the original mixture in the infrared indicated the presence of free hydroxyl groups, the mixture was converted to the trimethyl silyl ether derivative, and again subjected to GLC-MS. The gas chromatographic and mass spectral patterns (except that of peak 7) obtained were identical to those obtained for the sample prior to silylation. In the mass spectrum taken of the component eluting as peak 7 fragments were found at *m/e* 75, and 147, which corresponded to ions arising from the silyl ether moiety (Budziekiwicz *et al.*, 1967), as well as ions at higher masses *m/e* = 481 corresponding to loss of a methyl group from the original trimethyl silyl ether derivative. These data, in conjunction with other data obtained by GLC-MS (Figure 3) of the original material, indicated that peak 7 corresponded to nonacosanol. This structure was confirmed by a similar analysis of *n*-octacosanol. In addition, further confirmation was obtained by a comparison of the fragmentation pattern of the corresponding acetate derivative prepared from the sample and *n*-octacosanol.

The presence of a series of odd chain hydrocarbons was of interest since such hydrocarbons occurring in flower waxes are quite often of even chain length; although the presence of pentacosane and other high molecular weight hydrocarbons of odd chain length have been reported in wax isolated from *Citrus maxima* (Warth, 1956). The free alcohol components of flower waxes are also generally present as mixtures of higher alcohols, C<sub>20</sub>-C<sub>34</sub> (Warth, 1956). It is of interest that in the present study the major alcohol component which was detected was *n*-nonacosanol.

#### SUMMARY

The application of GLC-MS to the direct analysis of milligram amounts of material (in this case hydrocarbons and an alcohol) isolated from the flower of the rare tropical plant *Sesbania grandiflora* has been illustrated. A homologous series of hydrocarbons (C<sub>23</sub>-C<sub>31</sub>) were identified as was the alcohol *n*-nonacosanol.

#### LITERATURE CITED

- Budziekiwicz, H., Djerassi, C., Williams, D. H., "Mass Spectrometry of Organic Compounds," p. 476, Holden Day Inc., San Francisco, Calif., 1967.
- Eneroth, P., Hellstrom, K., Ryhage, R., *J. Lipid Res.* **5**, 245 (1964).
- Ryhage, R., *Anal. Chem.* **36**, 759 (1964).
- Shriner, R. L., Fuson, R. C., "Systematic Identification of Organic Compounds," p. 165, J. Wiley and Sons, New York, N. Y., 3rd ed., 1948.
- Stahl, E., "Thin-Layer Chromatography," p. 350, Academic Press, New York, N. Y., 1965.
- Warth, A. H., "The Chemistry and Technology of Waxes," pp. 313-22, Reinhold Publishing Corp., New York, 2nd ed., 1956.
- Watson, J. T., Biemann, K., *Anal. Chem.* **37**, 844 (1965).

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