Pak. j. sci. ind. res., vol. 34, nos. 7-8, July-August 1991

ALKANES AND ALCOHOLS IN CASSIA SEED OIL

M. Y. Raie and Shaihna Zaka

PCSIR Laboratories Complex, Lahore-54600, Pakistan

(Received November 13, 1990; revised July 14, 1991)

Three species known as *Cassia absus, C. fistula* and *C. occidentalis* of the family Leguminosae contain non-saponifiable material (1.8%), (1.6%) and (1.2%) respectively which on further separation showed hydrocarbons (43.1%), (42.3%), (51.2%) in addition to alcohols (41.3%), (39.2%), (30.9%) and unidentified material (15.6%), (18.5%), (17.9%) respectively. The hdyrocarbons ($C_{18:0}$—$C_{32:0}$) and alcohols ($C_{12:0}$— $C_{28:0}$) were identified and characterized by the application of Chromatography and spectrophotometry.

Key words: Hydrocarbons, Fatty alcohols, Cassia species.

Introduction

The Cassia species of the family Leguminosae are abundently available in Pakistan. The work on oils [1] of these species was carried out for the characterization of fatty acids. The work on non-saponifiable material has not been studied previously. Usually the non-saponifiable material consists of hydrocarbons, aldehydes, ketones, alcohols, sterols, carotenoids, fat soluble vitamins etc. Such compounds are present either in free state orin the form of esters. Previous worker [2-6] determined the non-saponifiable material, but no further investigation was carried out for the separation and characterization of hydrocarbons and alcohols by the application of chromatography and spectrophotometry. Now the presented work is undertaken on hydrocarbons and fatty alcohols of non-saponifiable material of these species for further and thorough investigation.

Experimental

Procurement of unsaponifiable material. The yellow coloured oil (4.80 gm), (4.00 gm) and (3.55 gm) was extracted with hexane by using Soxhlet out of (100 gm) crushed seeds of *C. absus, C. fistula* and *C. occidentalis* respectively. The oil of each species was saponified and the unsaponifiable matter (1.8 gm), (1.6 gm) and (1.2 gm) of these species in the same order was procured by diethyl ether.

Thin layer chromatography. Fifteen thin layer chromatogram (20 cm x 20 cm) of thickness (0.25 mm) were prepared by using silica gel (90 gm) and distilled water (140 cm^3). These were activated at 105° for 60 mins for the separation of hydrocarbons and other fractions out of (300 mg) of non-saponifiable material of *C. absus* by using hexane/ diethyl other solvent systems (17:3 v/v). The locating reagent 2, 7 dichlorofluorescein was used which under UV at 254 nm showed purple yellow coloured bands of hydrocarbons near the solvent front, alcohols and un-identified material at the base. These bands were scratched and hydrobonds (129.3 mg) were extracted with hexane whereas the material (170.7 mg) of alcohols including unidentified material at the base was extracted with diethyl ether. The same process was repeated to get hydrocarbons (126.9 mg) and (153.6 mg) out of the oil of *C. fistula* and *C. occidentalis* respectively. The alcohols including unidentified material for these two species were (173.1 mg) and (146.4 mg) respectively.

Acetylations of alcohols. The mixture of acetic anhydride (8 cm^3), pyridine (24 cm^3) and the polar material (100mg) containing alcohols and unidentified material of each species was stirred for 18 hrs at room temperature. The acetates of alcohols were extracted with diethyl ether (3 x 75 cm) after the addition of water (60 cm^3). The acetates were thoroughly washed to remove pyridine and acetic anhydride and were dried over anhydrous sodium sulphate prior to their recovery after the distillation of diethyl ether. The alcohol acetates were purified by thin layer chromatography under the above conditions and procedure. The alcohol acetates of *Cassia absus, C. fistula* and *C. occidentalis* were (72.6 mg), (67.9 mg) and (63.7 mg) respectively whereas, the unidentified material was (27.4 mg), (32.1 mg) and (36.3 mg) respectively.

Infrared spectrophotometry. The instrument (Beckmann - IR Model 54) was used for the identification of hydrocarbons and purified alcohol acetates. The hydrocarbons showed infrared absorption at 2860 cm^{-1} (CH_3stretch), 2940 cm^{-1} (CH_2 -stretch), 1380 cm^{-1} (CH_3 -bend), 1460 cm^{-1} (CH_3 bend) whereas the purified acetates of alcohols showed infrared absorption at 1240 cm^{-1} (C-O stretch of acetate), 1370 cm^{-1} (CH_3 bend), 1460 cm^{-1} (CH_2 bend), 1730 cm^{-1} (C=O stretch), 2840 cm^{-1} (CH_3 stretch) and 2930 cm^{-1} (CH_2 stretch).

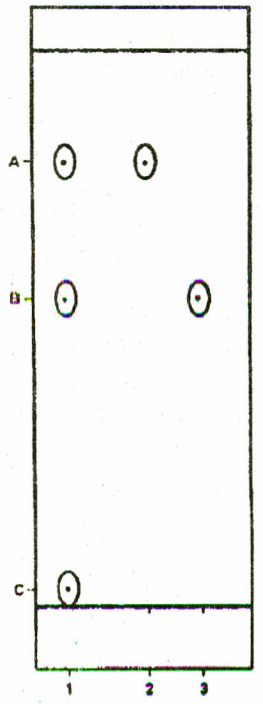
Gas liquid chromatorgaphy. A column (125.4 cm x 0.95 cm) was prepared by coating Apiezon L (5.0%) on diatomite "C" (80-100 mesh). The flow rate of nitrogen as a carrier gas 40 ml/min and temperature 200° for the identification of hydrocarbons and alcohols as alcohol acetates by the comparison of their retention times with the pure standard hydrocarbons and long chain alcohol acetates under the same conditions. The percentage of each hydrocarbon and alcohol

acetate was determined by calculating the peak area by the multiplication of height and the width at half height of the peak by the application of gas liquid chromatography (Pye Unicam 204 Series).

Results and Discussion

The oil was extracted by hexane [7] and saponified [8]. The non-saponifiable material was checked qualitatively by thin layer chromatography. The solvent system used was hexane: diethyl ether (17:3 v/v). It shows the presence of hydrocarbons near the solvent front and the polar material at the base. The hydrocarbons were separated quantitatively and confirmed by infrared spectrophotometery. The hydrocarbons were identified by the application of gas liquid chromatography by using a non-polar Apiezon L column (5.0%). The polar material at the base was checked up by infrared spectrophotometry which shows the presence of alcohols by absorption at 3600 cm^{-1} stretching. The polar material was acetylated, purified and checked by infrared spectrophotometry which indicated the presence of alcohols as acetates by absorption at 1240 cm^{-1} (C-O stretch of acetates). The alcohol acetates were separated quantitatively from the most polar and unidentified colouring material by thin layer chromatography and alcohols were identified by gas liquid chromatography. Further work on the unidentified material may be carried out. However, the qualitative check up of hydrocarbons, alcohols as acetates and unidentified material is shown in Fig. 1. The percentage of non-saponifiable material of *C. absus* is (1.8%) which is higher than (1.6%) and (1.2%) in *C. fistula* and *C. occidentalis* respectively. The alcohols are usualy present in free state or in esterified form mostly as cuticular wax in the plant kingdom. The waxy material was procured during the process of extraction. Anyhow, alcohols are produced as a part of non-saponifiable material after saponification of the oil.

The pure hydrocarbons separated by thin layer chromatography were identified by the use of gas liquid chromatography (Table 1). The *C. absus, C. fistula* and *C. occidentalis* show hydrocarbons (43.1%), (42.3%) and (51.3%) respectively. The *C. Occidentalis* shows high percentage as compared to other two species. All these three species contain hydrocarbons ($C_{18:0}$ -$C_{32:0}$) and the pattern of distribution of hydrocarbons in these species is almost the same. The non-saponifiable of *Cassia absus, C.fistula* and *C. occidentalis* also contain fatty alcohols (41.3%), (39.2%) and (30.9%) respectively. Alcohols of each species were acetylated, [9-10] purified and identified by chromatography (Table 2). Each species of Cassia has alcohols with same degree of unsaturation but the unsaturation is restricted to the C_{18} chain length. The total content of C_{18} is (27.1%), (21.5%) and (21.7%) for *C. absus, C. fistula* and *C. Occidentalis* respectively.



(1). Sample. (A) Hydrocarbons. (B) Alcohol acetates (C) Unidentified material (2) Standard hydrocarbon (Octadecane). (3). Standard alcohol (Octadecyl acetate).

Fig. 1. Separation of hydrocarbons, alcohol acetates and unidentified material by thin layer chromatography the solvent system used was hexane diethyl-ether (85:15).

TABLE 1. HYDROCARBONS OF THREE SPECIES OF CASSIA.

S. No.	Cassia species	$C_{18:0}$	$C_{19:0}$	$C_{20:0}$	$C_{21:0}$	$C_{22:0}$	$C_{23:0}$	$C_{24:0}$	$C_{25:0}$	$C_{26:0}$	$C_{27:0}$	$C_{28:0}$	$C_{29:0}$	$C_{30:0}$	$C_{31:0}$	$C_{32:0}$	$C_{33:0}$
1.	*C. absus*	1.5	21.2	3.5	9.7	4.3	11.6	1.7	5.5	Trace	10.8	Trace	16.4	1.3	7.5	1.2	3.8
2.	*C. fistula*	2.4	20.5	2.2	7.0	Trace	12.6	7.7	4.0	1.7	13.4	1.7	16.0	1.1	6.4	Trace	3.3
3.	*C. occidentalis*	1.1	23.2	2.3	11.4	3.8	12.3	Traces	4.4	0.9	13.6	0.8	13.2	Trace	8.2	1.5	3.3

TABLE 2. FATTY ALCOHOLS OF THEIR SPECIES OF CASSIA.

S.No.	Cassia species	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:0}$	$C_{22:0}$	$C_{24:0}$	$C_{26:0}$	$C_{28:0}$
1.	*C. absus*	Trace	0.7	8.3	7.7	12.3	7.1	0.9	20.4	24.4	11.6	6.6
2.	*C. fistula*	0.6	0.9	10.3	5.7	10.5	5.3	1.5	12.0	25.5	21.4	6.3
3.	*C. occidentalis*	0.6	1.0	8.5	6.1	9.1	6.5	1.0	20.9	28.5	10.4	7.4

The inter conversion of lipids under the process of oxidation/reduction is supported by the most popular theory [11] for the biosynthesis of fatty alcohols assumes that fatty acids and fatty alcohols are in revisible equilibrium and that the acids are produced by the normal denovo process. Thus the composition of fatty acids, hydrocarbons and alcohols of seed oils of *C. absus, C. fistula* and *C. occidentalis* give a clear picture as compared to the previous work for understanding the biosynthesis of these compounds in the plant kingdom under the natural phenomenon.

References

1. Shahina Zaka, Waheed Akhtar, Shafiq Ahmad Khan and M. K. Bhatty, Proc. Pak. Acad. Sci., **23** (2), 167 (1986).
2. Salamon and Seaber, *Perfumery and Essential Oil Record* (1934), Vol. 25, pp. 277.
3. Schimmel *et al.*, *Am. Perfumer* (1928), Vol. 23, pp. 527.
4. I. R. N. Chakravarti and D. Chakravarti, J. Sci. Ind. Res., **15C**, 86I (1956).
5. R. N. Chakravarti, D. Chakravarti and M. N. Mitra, Bull. Calcutta School Trop. Med., **3**, 163 (1955).
6. A. Wm. Johnson, J. Org. Chem., **23**, 1814 (1958).
7. *Official Methods of Analysis of the Association of Official Analytical Chemists* (AOCS Washington D.C., 1975), 12th ed.
8. R. J. Hamilton, M. Long and M. Y. Raie, Am. Oil Chem. Soc., **49** (5), 307 (1972).
9. R. J.Hamilton and M. Y. Raie, Chemistry and Physics of Lipids, **14**, 92 (1975).
10. M. Y. Raie, Shahina Zaka and M. Saleem, Fatte Seifen Anstrichmittel, **85** (8), 325 (1983).
11. D. M. Sand, J. L. Hehl and H. Schlenk, J. Lipid Res., **6**, 562 (1971).