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Chemical Constituents of *Dolichos lablab* (Field Bean) Pod Exudate

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Analysis of the *Dolichos lablab* pod exudate by combined gas chromatographic–mass spectrometric method is described. The exudate is found to consist of homologous fatty acids and their methyl esters—42 in all—from C-11 through C-24, including the odd carbon chain compounds. Apart from *trans*-2-dodecenoic and *trans*-2-tetradecenoic acids, which constitute the major percentage of the oil, other homologous Δ^2-enoic acids and saturated acids and esters are also found.

Legume seeds form an important part of food, especially in poorer countries, and serve as a source of dietary protein (Evans et al., 1978; Mahadevappa and Raina, 1978). Seed pods of many legumes exude oily substances of an usually sweet odor. The exudates may be end products of a metabolic process or they may act as a protective coating for the pods. Because of their oderiferous character, they may possibly play some role in plant–insect relationships also. As a prelude to this study, we undertook to identify the chemical constituents of *Dolichos lablab* pod exudate, and the results are reported here.

Dolichos lablab, commonly referred to as field bean, is a legume some varieties of which secrete the so-called fragrant oil on the surface of their pods. In India it is an important multipurpose legume crop used as pulse, vegetable, and forage. Whereas its dried beans serve as pulse, its tender pod with beans or the green beans alone serve as vegetable. Because of its abundant foliage, the crop is employed as an excellent fodder. Besides India, it is also cultivated as a forage crop in the United States, Hungary, Nigeria, and many other countries. When used as a vegetable, the Indian consumers show preference for varieties with fragrant oil. Our preliminary observation has indicated that the exudate, even in low concentration, attracts the important insect pest of this crop, *Adisura atkinsoni*, whose life cycle appears to depend on the life cycle of the field bean plant (Govindan, 1974).

EXPERIMENTAL SECTION

The gas chromatographic–mass spectrometric (GC–MS) analyses were performed on a Finnigan 3200 E automated GC–MS instrument using a 1.52 m 3% OV-17 on Supelcoport 60/80 column, with temperature programmed at 12 °C/min from 50 to 300 °C. To establish the identities of the compounds in the exudate, GC–MS of the authentic samples were run under the same instrumental conditions.

Preparative GC was carried out on a Varian Aerograph Model 90-P using a 3.05 m 10% SE-30 on Chromosorb W column. Infrared spectra were obtained on a Beckman IR-33 infrared spectrophotometer, using liquid films between sodium chloride plates.

Isolation of the Exudate. The exudate was obtained by wiping the surfaces of mature green pods, while they were still on the plants, grown on GKVK Campus farm of the University of Agricultural Sciences, Bangalore, with small pieces (about 3 × 2 cm) of Whatman No. 1 filter paper. Each piece of filter paper with the exudate absorbed on it was quickly transferred to a bottle of petroleum ether (bp 50–60 °C). When the bottle was full, it was well shaken and the petroleum ether solution was decanted. Then the filter paper pieces were washed with methanol. The petroleum ether solution and methanol washings were combined and filtered. The solvents were removed under aspirator pressure at 40–50 °C, leaving a pleasant smelling oil, which was used as such for all subsequent work.

Separation of Acids from Esters A in the Exudate. About 2 g of the exudate was dissolved in 100 mL of ether and the resulting solution was extracted with saturated sodium bicarbonate solution (4 × 40 mL). The bicarbonate extracts were combined and carefully acidified with 3 M sulfuric acid. The regenerated free fatty acids were extracted with ether, dried over magnesium sulfate and the solvent was flash evaporated (aspirator pressure, 40–50 °C). The earlier neutral fraction was also dried ($MgSO_4$) and the solvent removed on a flash evaporator (aspirator pressure, 40–50 °C), leaving an oily residue referred to here as esters A. The weight ratio of esters A to free acids in the exudate is about 1:2.

Esterification of the Acids. The fatty acids were converted into their methyl esters (henceforth called esters B here) by the method of Clinton and Laskowski (1948) as described by Hickinbottom (1962). A solution of about 0.5 g of the acid mixture, 2 mL of methyl alcohol, and 5 drops of concentrated sulfuric acid in 15 mL of ethylene dichloride was refluxed overnight. After cooling, the reaction mixture was washed successively with water, sodium bicarbonate solution, and again water and then dried ($MgSO_4$). The solvent was rotoevaporated under aspirator pressure at room temperature to obtain esters B.

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Table I. Ester Components of *Dolichos lablab* Pod Exudate

compound	concn, %[a]	diagnostic mass spectral peaks (m/e)
1. methyl undecanoate	0.5	200, 171, 169, 157, 143, 129, 87, 74, 59, 55
2. methyl 2-undecenoate	Tr[b]	c
3. methyl laurate	28.8	214, 285, 283, 271, 157, 143, 129, 115, 101, 87, 74, 59, 55
4. methyl 2-dodecenoate	2.8	c
5. methyl tridecanoate	4.5	228, 199, 197, 185, 143, 129, 87, 74, 55
6. methyl 2-tridecenoate	Tr	c
7. methyl myristate	25.0	242, 213, 211, 199, 185, 171, 157, 143, 129, 115, 101, 87, 74, 55
8. methyl 2-tetradecenoate	2.3	c
9. methyl pentadecanoate	1.3	256, 227, 225, 213, 199, 185, 171, 157, 143, 129, 115, 101, 87, 74, 55
10. methyl palmitate	8.0	270, 241, 239, 227, 213, 199, 185, 171, 157, 143, 129, 115, 101, 87, 74, 55
11. methyl heptadecanoate	1.2	284, 255, 253, 241, 227, 213, 199, 185, 171, 157, 143, 129, 87, 74, 55
12. methyl stearate	10.2	298, 269, 267, 255, 213, 199, 185, 143, 129, 87, 74, 55
13. methyl linoleate	2.1	294, 263, 220, 178, 165, 150, 135, 123, 109, 95, 81, 79, 74, 67, 59, 55
14. methyl arachidate	3.9	326, 297, 295, 283, 255, 241, 227, 199, 185, 171, 157, 143, 129, 87, 74, 57, 55
15. methyl 2-eicosenoate	0.7	c
16. methyl heneicosanoate	0.9	340, 309, 297, 283, 255, 241, 227, 213, 199, 185, 171, 143, 129, 87, 74, 55
17. methyl behenate	7.6	354, 323, 311, 269, 255, 199, 185, 143, 129, 87, 74, 55
18. methyl tricosanoate	Tr	368, 325, 312, 297, 283, 269, 255, 143, 129, 87, 74, 55
19. methyl lignoserate	Tr	382, 351, 339, 325, 312, 297, 283, 269, 255, 143, 129, 87, 74, 55

[a] The concentrations are only approximate as no calibration was done using standard mixture, and were obtained by weighing the pieces of paper under the chromatogram peaks (average of five xerox copies). [b] Concentrations less than 0.5% are considered as trace. [c] See Table II for mass spectral data.

Hydrogenation of Esters B. About 0.1 g of esters B in 8 mL of absolute ethanol was hydrogenated using Adam's catalyst under 3 atm pressure, in a Parr hydrogenating equipment. The hydrogenated esters were recovered by filtering off the catalyst and stripping the solvent on a flash evaporator (aspirator pressure, room temperature).

Preparation of Trimethylsilyloxy (TMSO) Derivatives. The TMSO derivatives of the olefinic esters were prepared following the procedure described by Capella and Zorzut (1968).

RESULTS AND DISCUSSION

The GC–MS of the crude exudate indicated that it is a fairly complex mixture of fatty acids and fatty acid methyl esters, which did not separate satisfactorily in GC, thus rendering it difficult to interpret the mass spectra. For correct identification of the components in the mixture, better separation was essential, which was achieved by converting the carboxylic acids into their methyl esters (Sonntag and Rini, 1968; Landone et al., 1976). However, since the exudate itself is a mixture of fatty acids and fatty acid methyl esters, for a better interpretation of the results, the separation of the esters from the acids was obviously useful, and this was achieved as described in the Experimental Section.

Identification of Esters A. The individual components and their composition in the esters A portion of the exudate are summarized in Table I. It is clear that esters A are methyl esters of mainly saturated fatty acids. The unsaturated esters are present in only small amounts. This is surprisingly in contrast to the acid components listed in Table II. The esters of the same chain length occur in almost the same proportion as the combined acid (as esters B) components of the corresponding chain length. This fact is brought into focus also by complete hydrogenation of the esters B, the results being presented in Table III for comparison.

All the saturated esters showed in their mass spectra, apart from the appropriate molecular-ion peaks, a base peak at m/e 74, peaks corresponding to M – 29, M – 31, and peaks attributable to the general formula $[(CH_2)_nCOOCH_3]^+$ where n = 2, 6, 10 ..., of which the peak corresponding to n = 2 (m/e 87) is the second most abundant in all these spectra. These are the characteristic mass spectral fragmentation patterns of methyl esters of long-chain fatty acids (Budzikiewicz et al., 1967; Ryhage and Stenhagen, 1963). The spectra also contained weak lines representing successive loss of methylene groups (mass unit 14) from the chain $[(CH_2)_nCOOCH_3$, n = 3, 4...]. These observations were confirmed by comparing these mass spectra with those of authentic esters taken under the same instrument conditions. Small amounts of unsaturated esters were also detected in this ester group and are included in Table I. Their identification was carried out by comparing their mass spectra with those of the corresponding compounds in esters B (Table II).

Identification of Acids (as Esters B). For the purpose of good separation and easy analysis the fatty acids were esterified as has already been described. The individual acids and their concentrations that occur in this

Table II. Acid Components of *Dolichos lablab* Pod Exude

compound	concn, %[a]	diagnostic mass spectral peaks of methyl ester (*m/e*)
1. undecanoic acid	0.5	*b*
2. 2-undecenoic acid	Tr	199 (M + 1), 167 (M − 31), 113, 87, 81, 74, 69, 55, 43, 41
3. lauric acid	13.1	*b*
4. 2-dodecenoic acid	20.3	213, 181, 138, 113, 87, 81, 74, 69, 55, 43, 41
5. tridecanoic acid	3.8	*b*
6. 2-tridecenoic acid	6.0	227, 195, 152, 113, 87, 81, 74, 69, 55, 43, 41
7. myristic acid	13.2	*b*
8. 2-tetradecenoic acid	17.5	241, 209, 166, 113, 87, 81, 74, 69, 55, 43, 41
9. pentadecanoic acid	1.0	*b*
10. 2-pentadecenoic acid	1.2	255, 223, 113, 87, 81, 74, 69, 55, 43, 41
11. palmitic acid	4.5	*b*
12. 2-hexadecenoic acid	2.3	269, 237, 184, 171, 152, 113, 87, 81, 74, 69, 55, 43, 41
13. heptadecanoic acid	0.9	*b*
14. stearic acid	4.1	*b*
15. linoleic acid	3.3	*b*
16. 2-octadecenoic acid	0.7	297, 265, 113, 87, 81, 74, 69, 55, 43, 41
17. nonadecanoic acid	0.5	312, 269, 255, 241, 227, 213, 199, 185, 171, 157, 143, 129, 101, 87, 74, 55
18. arachidic acid	2.3	*b*
19. 2-eicosenoic acid	Tr	325, 293, 113, 87, 74, 69, 55, 43, 41
20. heneicosanoic acid	0.6	*b*
21. behenic acid	2.9	*b*
22. tricosanoic acid	Tr	*b*
23. lignoseric acid	0.5	*b*

[a] The concentrations are only approximate, and were obtained by the method of triangulation. [b] See Table I for mass spectral data.

Table III. Hydrogenation Products of Esters B

compound	concn, %[a]
1. methyl undecanoate	1.1
2. methyl laurate	35.6
3. methyl tridecanoate	9.1
4. methyl myristate	32.3
5. methyl pentadecanoate	1.8
6. methyl palmitate	5.4
7. methyl heptadecanoate	0.7
8. methyl stearate	7.1
9. methyl nonadecanoate	0.5
10. methyl arachidate	2.2
11. methyl heneicosanoate	0.6
12. methyl behenate	2.8
13. methyl tricosanoate	Tr
14. methyl lignoserate	0.6

[a] Concentrations are approximate and were obtained by weighing the pieces of paper cut under the chromatogram peaks (average of five xerox copies).

portion of the exudate are recorded in Table II. It can be readily seen from the table that the major constituents in this group are α,β-unsaturated C-12 and C-14 acids. The ratio of saturated to unsaturated acids is about 1:1. The saturated esters of this group were identified in the same way as the esters A.

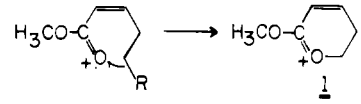
To obtain general information regarding the chain lengths of the various fatty acids present, the esters B were fully hydrogenated. The GC of the hydrogenated product was similar to that of esters A, showing essentially the same proportion (compare Tables I and III).

Table IV. Spectral Data of Methyl *trans*-2-Dodecenoate and Methyl *trans*-2-Tetradecenoate[a]

compound	infrared bands, cm^{-1}	mass-spectral peaks of TMSO derivatives (*m/e*)
C-12:1[2]	2970 (sh),[b] 2945 (s), 2870 (s), 1725 (s), 1655 (m), 1465 (m), 1430 (m), 1260 (m), 1190 (m), 1165 (m), 1120 (w), 1030 (m), 975 (m)	375 (M − CH_3), 336, 315, 285, 243, 234, 229, 163, 147, 130, 103, 83, 73
C-14:1[2]	2970 (sh), 2945 (s), 2870 (s), 1725 (s), 1655 (m), 1465 (m), 1430 (m), 1260 (m), 1190 (m), 1165 (m), 1120 (w), 1030 (m), 975 (m)	403 (M − CH_3), 359, 313, 257, 234, 147, 130, 103, 97, 83, 73

[a] See Table II for mass-spectral data. [b] sh, shoulder; s, strong; m, medium; w, weak.

The identification of the methyl esters of the unsaturated acids was accomplished through their very characteristic fragmentation pattern in their mass spectra (Budzikiewicz et al., 1967). Besides the appropriate molecular ion peak and M − OCH_3 ion peak, each spectrum showed, among others, a peak at *m/e* 87 more intense than the one at *m/e* 74 (in contrast to saturated analogues), and a fairly abundant peak at *m/e* 113. These characteristic peaks are associated with the typical fragmentation features of methyl esters of Δ^2-fatty acids, especially the ion of *m/e* 113, believed to be due to the cyclic ion 1 which

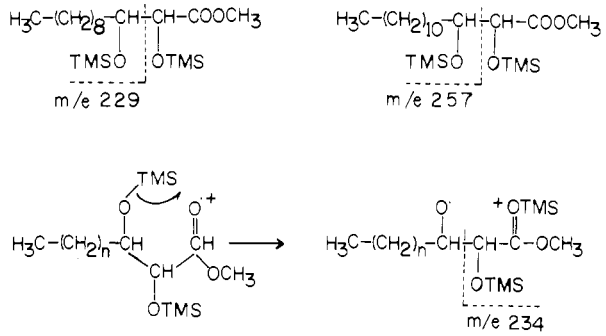


is highly diagnostic.

To confirm these structural assignments and to establish the geometry of the double bond, we isolated the two major methyl Δ^2-enoates (C-12:1[2] and C-14:1[2]) by preparative gas chromatography. The mass spectra of the pure isolated samples were rerun for comparison. The infrared spectra were very much similar to each other and were comparable to the reported spectrum of ethyl *trans*-2-dodecenoate (Creveling and Jennings, 1970). Each infrared spectrum shows a carbonyl band at 1725 cm^{-1} which signifies a marked low frequency shift from normal ester carbonyl absorption around 1740 cm^{-1}. In addition, a medium strong band at 1655 cm^{-1}, attributed to carbon–carbon double bond appears, which is not usually observed if the double bond is not terminal or is not adjacent to a polar group (Bellamy, 1958; Rao, 1963). Both these features can be explained if the double bond is in conjugation with ester carbonyl group.

Further support for the location of the double bond was obtained from the mass spectra of the trimethylsilyloxy (TMSO) derivatives of the two esters. The spectral characteristics of the two esters along with the mass spectral data of their TMSO derivatives are summarized in Table IV. Recently there have been many reports of the successful application of TMSO derivatives of unsaturated fatty acid esters using mass spectral analysis to locate the double bond position in the fatty acid chain (Capella and Zorzut, 1968; Dommes et al., 1976; Cavalli et al., 1978). The mass spectra of the TMSO derivatives of both C-12:1 and C-14:1 acid methyl esters show no

Scheme I



molecular ion peak, but an M – CH_3 peak due to loss of CH_3 radical from the trimethylsilyl group. In each spectrum the base peak appears at m/e 73 (trimethylsilyl group). In the case of the TMSO derivative of methyl dodecenoate the peaks at m/e 234 and m/e 229 are noteworthy. These peaks can be attributed to ions formed by fragmentation as depicted in Scheme I, in analogy with the mechanism proposed by Capella and Zorzut (1968) for TMSO derivatives of many other monounsaturated esters. The corresponding peaks for the TMSO derivative of methyl tetradecenoate appear at m/e 234 and m/e 257. The fragmentation ion of m/e 234 appearing in both cases strongly favors the conjugation of the double bond with the ester group. The position of the double bond thus established, it remains to settle its geometry. The evidence for this comes from the appearance of an absorption band at 975 cm^{-1} in the infrared spectra of both C-12:1[2] and C-14:1[2] esters. This band, which is supposed to arise from the out-of-plane bending mode, is a strong diagnostic feature of a trans geometry of the olefinic hydrogen atoms (Bellamy, 1958). All this infrared and mass spectral evidence indicates undoubtedly that we are dealing with *trans*-2-dodecenoic and *trans*-2-tetradecenoic acids. Because of the strong similarity of the mass spectra of the methyl esters of other monoenoic acids in the exudate to those of C-12:1 and C-14:1, it is reasonable to assume that they are also Δ^2-enoic acids with the configuration of the double bond being probably trans. The one and only nonconjugated dienoic acid found in the exudate was linoleic acid. Its presence was deduced by comparing the mass spectrum of its methyl ester with that of an authentic sample. It, however, amounts to only a small percentage of the total unsaturated acids.

CONCLUSION

The interesting and noteworthy features of the *Dolichos lablab* pod exudate are (1) the occurrence of continuous series of homologues of odd and even numbered straight carbon chain fatty acids from C-11 through C-24 without any omissions, (2) almost all the saturated acids in the series occur both as free acids and as their methyl esters, (3) most of the saturated acids are followed by their α,-β-unsaturated analogues, which occur mainly as free acids, (4) the major constituents of the mixture are *trans*-2-dodecenoic acid and *trans*-2-tetradecenoic acid. Though *trans*-Δ^2-enoic acids are found in nature (Creveling and Jennings, 1970), such a concentration of them and so many homologues as found in the present case is notably unusual. It is also to be recognized that the most common and widely distributed olefinic acids in natural fats like oleic, linolenic, palmitoleic etc. acids (Hilditch and Williams, 1964) are absent in the exudate. (5) The exudate probably contains some small amounts of glycerides since we could identify glycerol as its TMSO derivative after saponification of the exudate. However, we have not established the nature of these glycerides.

The function of the exudate in the life of the field bean plant is not known. It could be just an end product of the metabolic process, or it may be playing some other ecological role. However, we would like to record here that whereas it acts as an attractant for the insect pest of this crop, under laboratory conditions, for some other insects it acts as a repellent. We also noticed that the exudate acts as bactericide or bacteristat, which is in conformity with the fact that fatty acids and their derivatives do possess such properties (Rieth, 1976; Sumrell et al., 1978; Mazliak, 1963). Therefore we speculate that the exudate plays an ecologically important role and an understanding of its nature would perhaps be helpful in the pest management of the field bean crop.

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