

LIPID CONTENT AND COMPOSITION OF VESICLES OF A VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGUS

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ABSTRACT

The lipid, fatty acids, and sterol content of a pure preparation of the intramatrical vesicles of a *Glomus* sp. were determined. Lipid content of vesicles was 58.2% of the dry mass. The predominant lipid fraction was glycolipid and sphingolipid (44.5% of total lipid) followed by neutral lipid (25.5%) and phospholipid (24.2%). The fatty acids were examined by gas liquid chromatography. While 16:1 fatty acid was exceptionally high in the neutral and glycolipid fraction (60% and 51.2% of the total fatty acids), 18:2 was the predominant fatty acid in the phospholipid fraction (70.9%). The sterol content of the intramatrical vesicles was 5.8% of the spore dry mass (11.3% of total lipid). These results are compared to other lipid information available on the VAM and other fungi.

Key Words: fatty acids, *Glomus*, lipid, sterols, VAM, intramatrical vesicles.

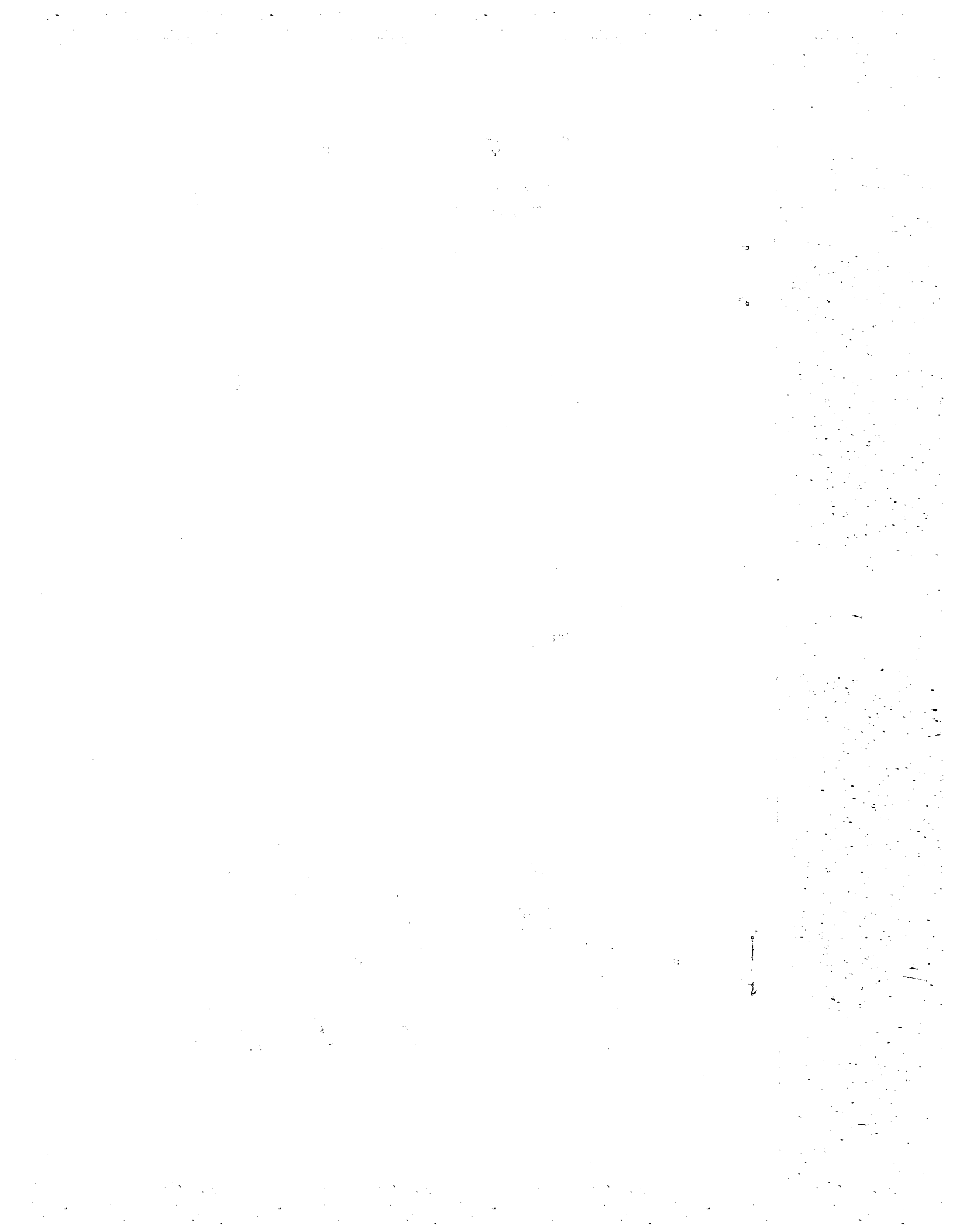
Since vesicular-arbuscular mycorrhizal (VAM) fungi cannot yet be grown in axenic culture, their lipid metabolism is yet not fully understood. Ectomycorrhizal fungi (mainly Basidiomycetes and Ascomycetes) store food as fungal carbohydrates (glycogen, mannitol, trehalose). VAM fungi, which are tentatively placed in the Zygomycota, accumulate lipid bodies in their thalli (Cox *et al.*, 1975). There are many reports of what are presumed to be lipid droplets in the hyphae and spores of VAM fungi (e.g., Mosse, 1959; Mosse and Bowen, 1968; Ho and Trappe, 1973). Electron microscopic examination of mycorrhizal roots for lipids revealed lipid droplets in vesicles of VAM fungi and in their intercellular and external hyphae (Cox and Sanders, 1974). Histochemical tests (Nemec, 1981) indicated that the main storage lipids in vesicles and hyphae of *Glomus etunicatum* Becker & Gerd. were neutral lipids, while phospholipids and glycolipids predominated in arbuscules.

Studies with ¹⁴C-compounds demonstrated the fate and distribution of photosynthates after translocation to the mycorrhizal fungi. Ho and Trappe (1973) reported that ¹⁴C-labelled photosynthates accumulated in lipid-filled chlamydospores of *Endogone (Glomus) mosseae* (Nicol. & Gerd.) Gerd. & Trappe. Bevege *et al.* (1975) found that much of the ¹⁴C-lipid fraction was concentrated in hyphae external to the roots of clover. When Lösel and Cooper (1979) fed labelled lipid precursors to onion seedlings, the label tended to accumulate in mycorrhizal roots.

Cooper and Lösel (1978) were the first to quantify lipids in mycorrhizal roots of onion, clover, and ryegrass; they found that mycorrhizal roots contained significantly more total lipids, triglycerides, and phospholipids than did non-infected roots. Nagy *et al.* (1980) also detected elevated concentrations of triglycerides and phospholipids in mycorrhizal citrus roots. Although the fungal component was assumed to be responsible for these elevated lipid levels, altered lipid concentrations in the plant tissues could not be ruled out.

Beilby and Kidby (1980a) were the first to study the lipid composition of isolated VAM fungal structures. They found that ungerminated chlamydospores of *Glomus caledonicum* (Nicol. & Gerd.) Trappe & Gerd. contained 45% total lipid, while Beilby (1980) found 45.5% lipid in ungerminated chlamydospores of *Acaulospora laevis* Gerd. & Trappe.

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higher in the vesicles, since these structures are thought to function as intramatrical storage organs. However, spores and mycelia of other fungi have also been reported to contain high amounts of polar lipids. Mycelia of the coelomycete, *Macrophomina phaseolina* Tassi (Goid.) contained very high levels of glycolipids (Wassef *et al.*, 1975).

The fatty acids of *Glomus* sp. vesicles were similar to those found in chlamydospores of *A. laevis* (Beilby, 1980) and *G. caledonicum* (Beilby and Kidby, 1980a). The unsaturated fatty acid 16:1 was exceptionally high in the neutral and glycolipid fraction (TABLE II). The polyunsaturated fatty acid, linoleic acid (18:2), was the predominant fatty acid (phospholipid fraction) in the vesicles of *Glomus* sp. This was not unexpected, as most Zygomycetes synthesize two other polyunsaturated fatty acids: α -linoleic 18:2, and γ -linolenic acid 18:3 (Wassef, 1977). There is evidence that to maintain the fluidity and flexibility of their membranes over a wide range of temperature, fungi adjust the degree of lipid unsaturation (Weete, 1981). Polyunsaturated fatty acids in lichens (another symbiosis involving fungi) may confer high flexibility on membranes at low temperatures, and are less susceptible to photooxidation at high daytime temperatures (Dertien *et al.*, 1977). The presence of high amounts of polyunsaturated fatty acids in VAM fungi may help to maintain their viability in nature.

We realize the shortcomings of the comparisons made in the foregoing discussion: we are comparing different structures of different taxa, perhaps at different points in their life cycle. Nevertheless, we believe that some trends emerge from our discussion. We hope that the paucity of research in this area, and the great need for a better understanding of the physiology of VAM fungi, will stimulate others to carry out similar investigations.

ACKNOWLEDGMENT

This work was supported by N.S.E.R.C. Operating grants to Dr. Bryce Kendrick and Dr. J. E. Thompson. We thank Dr. N. Lem for a critical review of the manuscript.

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These are remarkably high percentages, since the mycelial fungi listed by Weete (1980) contained an average of 17% lipid under favorable conditions of growth. Neutral lipids comprised over 95% of the total lipids in ungerminated chlamydo-spores of *G. caledonicum*. Triglycerides were the most abundant neutral lipids, with lesser amounts of diacylglycerides and monoglycerides. The major fatty acids were 16:1, 16:0, and 18:1. These comprised 63% of total fatty acids. Such high levels of 16:1 are unusual in fungi (Wassef, 1977).

As the foregoing literature review shows, the lipids of VAM fungi have so far been quantified only in their extramatrical spores. This paper provides the first report on the lipid content and composition of the intramatrical vesicles of an undescribed *Glomus* species, which we extracted from the root tissue by a new technique (Jabaji-Hare *et al.*, 1984).

MATERIALS AND METHODS

Preparation of VAM inoculum.—

(i) *Origin of VAM inoculum.* A culture of an unnamed species of *Glomus* (Herb. DAOM 181602) supplied by Drs. A. Fortin and V. Furlan, Université Laval, Ste. Foy, Québec, formed vesicles in the host roots, but no extramatrical spores (Plenchette *et al.*, 1981). Biochemical characterization of this species was possible only if the intramatrical vesicles, present in densities of up to 500 per cm of root, could be extracted.

(ii) *Inoculum production.* Inoculum production and multiplication was carried out in roots of leek (*Allium porrum* L.). Leek seeds were surface-sterilized with 0.5% sodium hypochlorite solution for 45 min, rinsed in sterile distilled water, then sown in sterile vermiculite. After 21 da, seedlings were transplanted to 10 cm plant pots filled with turface (Plant Products Co. Ltd., Bramalea, Ontario, Canada). One gram of leek roots previously colonized by the fungus, was surface-sterilized by the method of Mosse (1962), then layered in the top 5 cm of surface. All pots were maintained in a controlled environment chamber at night/day temperatures of 18 and 24 C, 80% relative humidity, and illumination of $390.60 \mu\text{E s}^{-1}\text{m}^{-2}$ with a photoperiod of 16 h. The seedlings were watered daily with distilled water, and each pot received 25 ml of Long Ashton solution per week. During the last month of growth, soil cores (2×10 cm) were removed from each pot to check for possible contamination by other fungi and soil animals. After six months, the plants were removed from the growth medium and the roots were washed thoroughly with distilled water to remove soil particles and debris.

(iii) *Isolation of vesicles.* Clean, colonized roots were macerated in a mortar and pestle, and homogenized in a Polytron type PT 20ST (Brinkman Instruments, Rexdale, Ontario, Canada). The homogenate was filtered through cheesecloth, adjusted to 15 mM CsCl, then centrifuged in a discontinuous sucrose gradient prepared as follows: 30 ml of filtrate was layered on top of 8 ml of 1.3 M sucrose—15 mM CsCl. The gradients were centrifuged at $82,000 \times g$ for 180 min at 4 C. The interface zone, in which the vesicles were concentrated was collected, diluted with 0.05 M NaHCO_3 at pH 7, and washed twice by centrifugation at $20,800 \times g$ for 45 min at 4 C. Virtually no root material was present in this fraction. This meant that our lipid analyses were not affected by plant components. The pellet was freeze-dried for 48 h then weighed and stored under nitrogen at -20 C. (see Jabaji-Hare *et al.*, 1984).

Preparation of lipids for analysis.—

(i) *Lipid extraction.* The isolation procedure described above was carried out six times, yielding an average of 46,000 vesicles per extraction. The freeze-dried vesicles from each isolation procedure were placed in a Braun bottle with 10

All glassware was washed thoroughly with 5% Decon and rinsed exhaustively with deionized water before use. Solvents were of analytical grade and were glass-distilled.

RESULTS

The total lipid content of *Glomus* vesicles was 58.2% (49.5–67.0) of the total dry mass. Sterols represented 5.8% (3.0–8.4) of total dry mass (11.3% of total lipid content).

The most abundant lipid class was glycolipid, representing 44.5% of the total lipid in the vesicles, while neutral lipids represented 26%, and phospholipids 24%. TABLE I expresses results in terms of total dry mass.

TABLE II presents the composition of fatty acids from neutral and polar lipid classes. Fatty acids ranged from 14:0 to 18:3. The proportion of total fatty acids in the neutral lipids and the polar lipids was similar. However, the fatty acid profiles of the three fractions were not identical. Analysis of the neutral lipid fatty acids showed that the most abundant fatty acid was 16:1 (60%), with the other predominant fatty acids being 18:1, 18:2, and 16:0. The profile of fatty acids in the glycolipid and sphingolipid fraction was similar except that 14:0 was present in trace amounts. The phospholipid fraction showed a distribution of fatty acids ranging from 16:0 to 18:3, with 18:2 (71%) being the principal fatty acid, followed by 16:0 and 18:1. Longer-chain fatty acids, such as C 20, saturated or unsaturated, were not detected in any of the three fractions.

DISCUSSION

The results reported above are the first quantifications of the lipid content and composition of intramatrical vesicles of a VAM fungus, and were made possible by a technique involving maceration of colonized roots followed by concentration of vesicles by centrifugation on a sucrose gradient (Jabaji-Hare *et al.*, 1984). Vesicles of *Glomus* sp. contained 58% (49.5–67.0) lipid. Most mycelial fungi so far investigated contain an average of 17%, and the lipid content of non-VAM fungal spores ranges from 1% to 35% (Weete, 1980). From the very limited amount of published information available on lipid content of extramatrical chlamydospores of VAM fungi (Beilby, 1980; Beilby and Kidby, 1980a), it appears that the intramatrical vesicles of *Glomus* sp. have a higher lipid content than the ungerminated chlamydospores of either *Acaulospora laevis* (45.5%) or *Glomus caledonicum* (45%).

The sterol content of *Glomus* sp. intramatrical vesicles was 5.8% of the total spore mass, which is much higher than the sterol content reported for either *A. laevis* (0.16% of spore mass; Beilby, 1980), or *G. caledonicum* (0.39% of the spore mass; Beilby and Kidby, 1980b). It is also higher than the range reported for some mucoralean fungi (trace amounts to 0.025% of mycelial dry weight; McCorkindale *et al.*, 1969). Weete *et al.* (1973) found that sterol levels in *Rhizopus arrhizus* (Mucorales) were 0.53%, 0.11%, and 0.2% of the total dry mass at 48 h, 96 h and 144 h growth, respectively. These values represented 3.46%, 1.88% and 9.1% of total lipids (total lipid values were 15.3%, 5.8% and 2.2%, respectively). In *Glomus* sp., which is tentatively placed in the Zygomycota (Benjamin, 1979), sterols account for 11% of the total lipids. Investigation of the nature of the individual sterol components is warranted.

The glycolipid-containing fraction in vesicles of *Glomus* sp. (TABLE I), expressed as a percentage of total lipid content, was exceptionally high compared to both the neutral and phospholipid fractions. Neutral lipid was expected to be

volumes of chloroform-methanol (1:1, v/v) and 10 volumes of 0.1 mm microglass beads. The cells were milled in a Braun apparatus for one min, and the contents were checked microscopically to ensure that all spores had been fractured.

The vesicle mixture was transferred into 125 ml screw-capped flasks, sealed under nitrogen, and subjected to Pedersen's (1962) lipid extraction method, modified as follows. The flasks were shaken for 3 h on a rotary shaker at room temperature and the contents centrifuged at $10,000 \times g$ on an HB-4 rotor for 10 min. After centrifugation, the solvent was placed in a round-bottomed flask, sealed under nitrogen and stored at -20 C, while the pellets were resuspended with 10 volumes of chloroform-methanol (1:1, v/v) and shaken for two further 3-h extractions. The solvent from the three successive extractions was pooled and dried down on a rotary evaporator at 37 C. The pellets were discarded. The crude lipid was dissolved in 10 ml of chloroform-methanol (2:1, v/v), mixed with 2 ml of 0.7% NaCl, and centrifuged for 5 min in a clinical centrifuge. The upper phases obtained were removed as completely as possible with a pipette and discarded, while the lipid sample was collected and dried on a rotary evaporator drier at 37 C. The lipid extracts, redissolved in 1.2 ml of chloroform-methanol (2:1, v/v), were transferred into Teflon-capped test tubes, and dried down in a vacuum chamber for at least 30 min. The lipid extract of each replicate was sealed under a stream of nitrogen at room temperature and stored in a freezer at -20 C.

(ii) *Lipid fractionation.* Three of the six replicates were fractionated and purified into three lipid classes by silica gel chromatography by the method of Rouser *et al.* (1976). The total lipid sample, dissolved in 1 ml chloroform, was applied to a silica gel 60 column, 5×1.1 cm, and the three fractions were eluted with 40 ml chloroform (neutral lipids), 140 ml acetone (glycolipids and sphingolipids) and 40 ml methanol (phospholipids).

Each fraction was rotoevaporated approximately to dryness at 37 C, and was then completely evaporated to dryness under nitrogen in a Teflon-screw-capped test tube.

(iii) *Fatty acid methyl ester preparation.* Preparation of fatty acid methyl esters from each of the lipid classes was carried out according to Morrison and Smith (1964). One mg of internal standard, heptadecanoic acid (C17) was added to each lipid fraction and dried down under nitrogen. Boron trifluoride (14%) in 0.5 ml methanol (Sigma Chemical Co.) was added to each tube and heated at 100 C for 10 min, then 0.4 ml of hexane and 1.1 ml of methanol were added and heated at 100 C for 30 min. The tubes were cooled and the esters extracted from the mixture with 2 volumes of hexane, then 1 volume of water, vortexed briefly, and centrifuged until both layers were clear. The top hexane layer was transferred to a 3 ml GC vial with septum cap (Teflon-laminated septum) previously rinsed with hexane. All tubes were sealed under nitrogen.

(iv) *Gas-liquid chromatography.* Fatty acid methyl esters were analyzed on a Perkin-Elmer Sigma 3B gas chromatograph with an FID detector using a 180×0.2 cm glass column packed with 10% DEGS on Supelcoport 80/100 mesh. The carrier gas was nitrogen at 25 ml/min flow, inlet 75 psi, inlet temperature 250 C, oven temperature 190 C, and detector temperature 250 C. Peaks were recorded by a PE Sigma 15 chromatograph Data Station, and the mass of the methyl esters calculated using an internal standard method based on relative mass response to the detector. Peak identification was made using fatty acid methyl ester standards and comparing them to the peaks in the sample. The individual fatty acids were calculated in mg per mg dry mass.

Total sterol extraction.—Total sterols were extracted from vesicles of *Glomus* sp. according to the method of Keller *et al.* (1969). Eight mg of total lipid extract

TABLE I
FRACTIONATION OF LIPIDS INTO CLASSES

	% of total lipids ^a	% of total dry mass of vesicles
Neutral lipid	25.5 (8.3-47.3) ^b	16.77
Glycolipid and sphingolipid	44.5 (23.1-67.0)	28.32
Phospholipid	24.2 (11.1-40.1)	15.91
Per cent recovery	94.2	

^a Mean based on 3 determinations.

^b Numbers in parentheses indicate asymmetrical 95% confidence limits using the arcsine transformation (Zar, 1974).

were dissolved in 8 ml of acetone-ethanol (1:1, v/v). Four ml of 0.5% w/v of digitonin dissolved in ethanol-water (1:1, v/v) was added to the mixture and stored at room temperature in the dark for 24 h. The solution was centrifuged at moderate speed for 10 min, after which the supernatant was discarded. The precipitate was washed twice with 6 ml of anhydrous diethyl ether-acetone (1:1, v/v); and centrifuged for 10 min after each washing. The precipitate was dried under a stream of nitrogen. The sterol-digitonin complex was redissolved in 2 ml pyridine, heated for 1 h, and stored at room temperature for 24 h. The digitonin was precipitated with 25 ml of diethyl-ether and the solution was centrifuged at 10,000 × g in an Hb-4 rotor for 30 min. The supernatant, which contained extracted sterols, was decanted into a round-bottomed flask and rotoevaporated to dryness, after which the dry weight of the sterol was recorded.

TABLE II
FATTY ACID COMPOSITION OF LIPID FRACTIONS OF *Glomus* SP. VESICLES

Fatty acid	Per cent of total fatty acids ^a		
	Neutral lipid	Glycolipid and sphingolipid	Phospholipid
14:0	ND ^b	t ^c	ND
16:0	8.3 (5.7-11.5) ^d	13.6 (10.2-17.3)	17.2 (8.7-27.6)
16:1	60.0 (50.2-69.2)	51.2 (37.1-65.3)	ND
18:0	ND	t	ND
18:1	19.4 (16.0-22.9)	19.1 (15.1-23.4)	10.8 (6.5-15.8)
18:2	12.3 (8.9-12.3)	13.9 (8.7-18.0)	70.9 (63.3-78.1)
18:3	ND	ND	t
20:0	ND	ND	ND
Total fatty acids ^e	25.5	17.7	24.2

^a Numbers are means based on 3 determinations.

^b ND, not detected.

^c T, trace (less than 1.0%).

^d Numbers in parentheses indicate asymmetrical 95% confidence limits using the arcsine transformation (Zar, 1974).

^e Numbers represent total fatty acids as per cent of different classes.