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FATTY ACID METHYL ESTERS IN PHOTOSYNTHETIC BACTERIA

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Key Word Index—*Rhodopseudomonas spheroides*; *Rhodospirillum rubrum*; Athiorhodaceae; photosynthetic bacteria; fatty acid methyl esters.

INTRODUCTION

Methyl esters of long chain fatty acids have been reported for corn pollen [1], chlamydospores of *Ustilago maydis* [2] and the sporangiophores and mycelia of *Rhizopus arrhizus* [3]. In addition, the biosynthesis of methyl esters of long chain acids has been demonstrated by a soluble enzyme from *Mycobacterium phlei* with the methylating agent being *S*-adenosylmethionine [4]. In the photosynthetic bacteria, the bacteriochlorophyll and specific proteins play the main role in chromatophore formation [5], but the lipid composition could be essential to the activity of some membrane enzymes [6].

RESULTS

Cells from *Rhodospirillum rubrum* wild type FR_1, blue green mutant FR_1 VI and *Rhodopseudomonas spheroides* wild type (NCIB No. 8253) were grown for 72 hr anaerobically in the light as described previously [7, 8]. The bacteria (about 10 g dry wt) were extracted according to the procedure used by Laseter *et al.* [2]. The cells were first extracted overnight in a mixture of $CHCl_3$–C_6H_6 (3:1) under nitrogen with continuous stirring. After filtration of the organic solvents, a second extraction was conducted using three times the same volume of petrol (bp 40–60°). The organic extracts were combined, evaporated to dryness under vacuum, taken up in petrol and chromatographed on a column of alumina (Woelm, grade I). The column was eluted successively with petrol, C_6H_6 and MeOH. The C_6H_6 fraction containing the esters was evaporated to dryness under vacuum, taken up in petrol and chromatographed on a column of neutral alumina (Grade I). The fraction eluted with 8% Et_2O in petrol was analyzed further.

The GC–MS instrument used had a glass column (1.5 m × 3 mm), packed with 10% SP 2340 on chromosorb WAW, 100/120 mesh. The temperature was 170°. The carrier gas was helium at 30 ml min^{-1}. The acceleration voltage was 3500 V and the energy of electrons was 70 eV. The esters encountered in these bacteria could not be identified by conventional GLC by using the retention times of standard saturated and monounsaturated fatty acids methyl esters (14:0, 16:0, 18:0 and 16:1, 18:1).

In *Rhodospirillum rubrum* FR_1 and FR_1 VI, the major free methyl esters were found to be branched ones. Thus methyl substitution at position 2 was unambiguously recognized by the intense ions corresponding to *m/e* 88 (base peak) and *m/e* 101. The ions at M-15 have a very low abundance in all cases and the fragmentation pattern for the hydrocarbon chains was the same as that already described for similar branched methyl esters [9]. We obtained the following parent ions M^+ 270, M^+ 284 and M^+ 298 which corresponded respectively to methyl-2-methylpentadecanoate, methyl-2-methylhexadecanoate and methyl-2-methylheptadecanoate.

These esters were saponified using 10% KOH in EtOH. After acidification of the aq. phase, the acids were extracted with Et_2O and methylated with diazomethane. The methyl esters obtained were purified on a neutral alumina column and then analyzed by GLC as described above. There was a good agreement between the retention times of the naturally occurring methyl esters obtained from the bacteria and the methyl esters obtained after saponification and methylation (9, 12 and 16 min respectively for the C_{16}, C_{17} and C_{18} branched fatty acids methyl esters).

It is to be noted that all these fatty acid methyl esters were present in extremely low quantities (never exceeding 10 μg per g dry wt).

By contrast, no fatty acids methyl esters were found in *Rhodopseudomonas spheroides* using the same experimental procedures.

At this time, on a theoretical basis, it seems difficult to exclude the occurrence of straight fatty acid methyl esters in traces in *Rhodospirillum rubrum* during a certain stage of the growth phase. Most of the branched fatty acids that have been identified from bacteria have proved to be *iso*- or *anteiso*-branched methyl compounds, but in many cases the branching is still unknown. Two branched fatty acids (C_{15} and C_{17}) have been found in the fatty acids of the bound lipids from *Rhodomicrobium vannielii* [10]. To our knowledge, our results provide the first evidence of naturally occurring fatty acid methyl esters in Athiorhodaceae. However further studies are needed to understand the biosynthesis and biological role of these branched methyl esters in photosynthetic bacteria.

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IRIDOID GLUCOSIDES OF *WENDLANDIA FORMOSANA*

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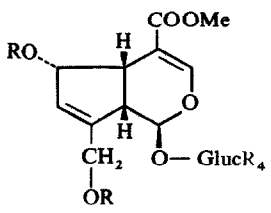
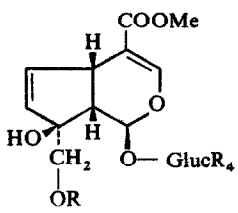
Key Word Index—*Wendlandia formosana*; Rubiaceae; iridoid glucosides; gardenoside; methyl deacetylasperulosidate; tarennoside; geniposidic acid.

Wendlandia formosana Cowan was collected in Ishigaki Island (Okinawa Pref.) on Aug. 27, 1974 and identified by Mr. G. Murata of Faculty of Science, Kyoto University. A voucher sample (Y. Takeda, Y. Ikeshiro & H. Nishimura No. 8) is deposited in the herbarium of the Institute of Botany, Faculty of Science, Kyoto University (KYO), Kitashirakawaoiwake-cho, Sakyo-ku, Kyoto, Japan.

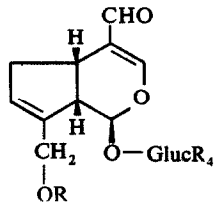
Air dried leaves (875 g) were extracted with hot MeOH. The extract was evaporated *in vacuo* and extracted with H_2O. After washing with EtOAc, the aq. extract was chromatographed on charcoal with H_2O–MeOH as eluent with increasing MeOH content. The 50% MeOH eluate gave upon TLC (Si gel, $CHCl_3$–MeOH 8:2) spots corresponding to methyl deacetylasperulosidate (1) (R_f 0.29), tarennoside (3) (R_f 0.17) and gardenoside (2) (R_f 0.13). Geniposidic acid (4) was also detected by TLC (Si gel containing 0.25% H_3PO_4, $CHCl_3$–MeOH 8:2, R_f 0.26). This eluate gave, on evaporation, 9.3 g of residue. A portion (0.92 g) of this residue was acetylated (Ac_2O–Py) and the product was chromatographed on Si gel with $CHCl_3$–MeOH as eluent. Fractions eluted with $CHCl_3$–MeOH (99.5:0.5) gave Acetate-1 and Acetate-3, while fraction eluted with $CHCl_3$–MeOH (99:1) Acetate-2: (a) Acetate-1 (47 mg), an amorphous powder, $[\alpha]_D^{30}$ + 34.8° ($CHCl_3$, c = 1.09) (lit. [1], $[\alpha]_D^{24}$ + 38.1° ($CHCl_3$, c = 0.84); lit. [2], $[\alpha]_D^{16}$ + 51.3° (EtOH, c = 0.78)); ν_{max}^{KBr} cm^{-1}: 1740, 1710, 1630; PMR ($CDCl_3$) δ: 1.93–2.10 (6 × OCOMe), 2.63 (1H, *m*, 9-H), 3.24 (1H, *m*, 5-H), 3.73 (3H, *s*, COOMe), 6.07 (1H, *m*, 7-H), 7.57 (1H, *d*, J = 1.5 Hz, 3-H). (Found: C, 53.04; H, 5.66. Cacld. for $C_{29}H_{36}O_{17}$: C, 53.04; H, 5.52%). Acetate-1 was identical to an authentic sample of methyl deacetylasperulosidate hexaacetate (= daphylloside pentaacetate) [1, 2] according to their IR and PMR spectra.



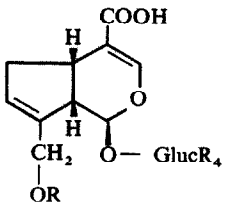
1: R=H
Acetate-1: R=COMe



2: R=H
Acetate-2: R=COMe



3: R=H
Acetate-3: R=COMe



4: R=H
Acetate-4: R=COMe

(b) Acetate-2 (232 mg), an amorphous powder, $[\alpha]_D^{30}$ − 97.6° ($CHCl_3$, c = 0.82) (lit. [1], $[\alpha]_D^{23}$ − 104.5° (MeOH, c = 0.22)); ν_{max}^{KBr} cm^{-1}: 3450, 1745, 1710; PMR ($CDCl_3$) δ: 1.91–2.11 (5 × OCOMe), 4.35 (1H, *d*, J = 2.0 Hz, 1-H), 5.70 (1H, *dd*, J = 6.0, 1.0 Hz, 7-H), 6.27 (1H, *dd*, J = 6.0, 2.5 Hz, 6-H), 7.32 (1H, *d*, J = 1.5 Hz, 3-H). (Found: C, 52.57; H, 5.52. Calcd. for $C_{27}H_{34}O_{16}$: C, 52.77; H, 5.58%). Acetate-2 was identical to an

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‡ The reported value of opposite sign [2] was found to be erroneous.