linked to the pyrrolic nitrogen[10]. This unit was shown to be $CH_2CH(CH_3)_2$, on the basis of spin-decoupling experiments: irradiation at δ 1.79 simplified the doublet at δ 3.12 into a singlet and caused collapse of the doublet at δ 0.63 to a sharp singlet.

The spectral data of **III**, considering those of molliorin-a, led us tentatively to assign structure **III** to the compound under investigation. Confirmatory evidence for this structure was provided by synthesis: condensation of scalaradial (**IV**)[11] with 2-methylpropylamine gave **III** in good yield, identical in all respects with the natural product.

Molliorin-c represents another example of mixed biogenesis and may be formally considered to be derived by a combination of a sesterterpenoid moiety and the isobuthylamine arising from valine by loss of CO_2.

The effect of γ-irradiation on soil enzyme stability*

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Summary. Arylsulphatase, β-1,3 glucanase, phosphatase and urease responded differently to γ-irradiation (5–50 Mrad) in air-dried and moist soils. In all instances phosphatase was the most stable. The variability between enzymes may be due to inherent biochemical and stuctural characteristics or to their location within the soil microenvironment.

The value of γ-irradiation in the study of soil microbiology and biochemistry has been discussed at length by Cawse[1] and McLaren[2]. A major *differential advantage* of this technique is that doses of ~ 5 Mrad can eliminate microbial proliferation whilst still allowing colloid-bound enzymes to function. Higher levels of irradiation will progressively denature soil enzymes. The protective characteristics of soil organic and inorganic colloids on extracellular enzymes has been reviewed by many[3–5], and includes resistance to temperature extremes, storage, and proteolysis as well as irradiation. There is little doubt that the accumulated and persistent soil enzyme fraction is crucial to the mineralization of organic matter. The work reported in this paper illustrates the differential stability of 4 soil enzymes when subjected to a range of γ-irradiation doses. In addition, some suggestions are made to account for the differences.

Materials and methods. A silt loam soil (< 2 mm) was used for all experiments. Its characteristics, fully described elsewhere[6], were: sand 16%; silt 64%; clay 20%; organic matter 5.4%; c.e.c. 14.8 mEq. 100 g soil^{-1}; pH 5.4; w.h.c. 0.72 ml · g soil^{-1}.

Soil samples (25 g) either air-dried or at field wetness (29% w.h.c.) were sealed in polyethylene bags and subjected to 5, 10, 15, 20 or 50 Mrad doses of γ-irradiation (approx. 4 Mrad · h^{-1}) at the AERE Harwell Fuel Pond Assembly. Prior and subsequent to irradiation soils were stored at 4 °C. Control soils were also sealed and refrigerated but did not receive irradiation treatment.

Arylsulphatase and phosphatase were assayed using p-nitrophenyl ester substrates[7]; β-1,3 glucanase using laminarin[8]; and urease after the method of Pettit et al.[9] but using 0.5 M tris-maleate buffer (pH 7.0) and adding 0.5 ml $AgSO_4$ (10 mM) to terminate the reaction. All the activities plotted in figures 1 and 2 at the means of at least 3 replicates, SD: arylsulphatase 2.5%; β-1,3 glucanase 6.5%; phosphatase 6.1%; urease 3.0%.

Results and discussion. From figures 1 and 2 it can be seen that in both the dry and wet soil phosphatase was the most resistent enzyme to γ-irradiation. From this data the levels of irradiation required to induce a 90% loss in activity were a) in the dry soil: phosphatase 48 Mrad; urease 19.5 Mrad; β-1,3 glucanase 18 Mrad; arylsulphatase 14 Mrad; b) in the wet soil: phosphatase 29 Mrad; β-1,3 glucanase 15 Mrad; arylsulphatase 9 Mrad; urease 7 Mrad. Following 50 Mrad treatment 7.5% of the phosphatase activity survived in the dry soil (next best was urease with 2.7%); 3.1% in the wet soil (all others zero). Ramirez-Martinez and McLaren[11] found that phosphatase was inactivated more rapidly in wet than dry soil and it is well known that the radio-sensitivity of microorganisms as well as enzymes generally increases in wet soil[12] due, in part, to the reactive free radicals (OH, H, HO_2) produced when water is ionized[13,14]. Skujins et al.[10] have described an inactivation coefficient (k) for enzymes: $N/N_0 = e^{-kD}$ where N = activity at irradiation dose D and N_0 = activity of nonirradiated soil. Using a graphical representation of equation (1) the computed values of k in dry soil were arylsulphatase 0.031 (correlation coefficient r = 0.99); β-1,3 glucanase, 0.020 (r = 0.96); phosphatase 0.009 (r = 0.99); urease 0.024 (r = 0.89). The equivalent values in wet soil were: arylsulphatase 0.049 (r = 1.0); β-1,3 glucanase 0.027 (r = 1.0); phosphatase 0.019 (0.97); urease 0.042 (0.99).

$$\log_{10}\frac{N}{N_0} = \frac{-k}{2.303} D \qquad (1)$$

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Skujins et al.[10] also noted the resistance of phosphatase to irradiation (37% survived 19 Mrad) but recorded an inactivation coefficient in dry soil different from our figure (i.e. 0.05).

The reasons for the differential susceptibility of the 4 enzymes are not resolved by these observations. However, it is pertinent to suggest that one or more of the following factors have relevance.

a) The inherent structural and chemical differences between the enzymes.

b) The protection afforded by the enzymes extracellular location. Urease, with its soluble, low molecular weight substrate may function from within the soil organo-mineral complex[15], and be somewhat less exposed to irradiation. β-1,3 glucanase, on the other hand, may be associated with the outer surfaces of the colloidal organic matter.

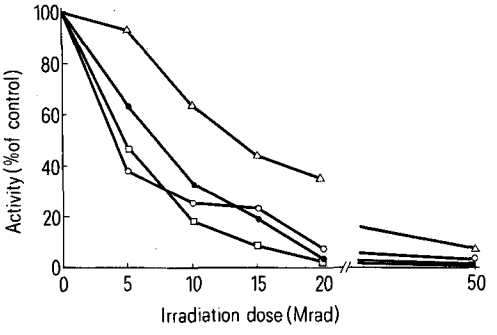

Fig. 1. The effect of γ-irradiation of dry soil on the activity of arylsulphatase (□), β-1,3 glucanase (●), phosphatase (△) and urease (○).

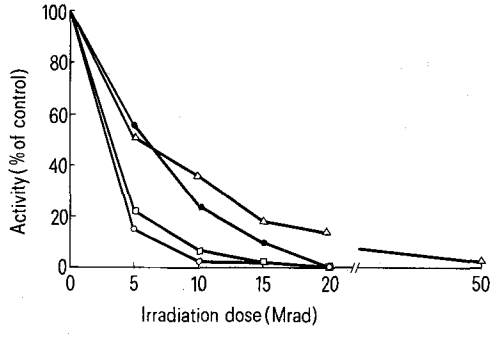

Fig. 2. The effect of γ-irradiation of wet soil on the activity of arylsulphatase (□), β-1,3 glucanase (●), phosphatase (△), and urease (○).

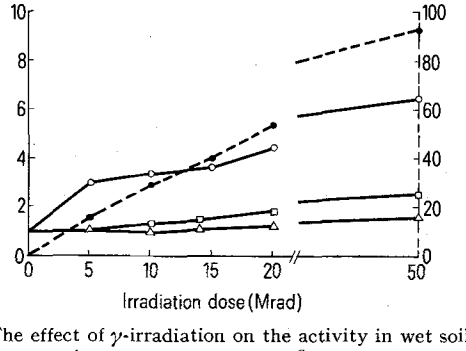

Fig. 3. The effect of γ-irradiation on the activity in wet soils of no-substrate controls: arylsulphatase (□); β-1,3 glucanase (●); phosphatase (△) and urease (○). Vertical axes represent the increase in level of activity as a factor of the control for arylsulphatase, phosphatase and urease (left hand axis) and β-1,3 glucanase (right hand axis).

c) Enzymes may be released from damaged cells during and after irradiation. The magnitude of this will be influenced by the type of microbial cell, the predominant location of the enzymes within that cell, and the level of irradiation. Periplasmic enzymes (e.g. phosphatase) may be more susceptible to leakage than are cytoplasmic enzymes (e.g. arylsulphatase and urease). If the released enzymes are not immediately denatured, irradiated soils may even show an increase in activity[16,17].

d) Irradiation may differentially change the permeability of intact cell membranes (or selectively eliminate transport mechanisms) thus allowing some substrates to enter the cell and excluding others. Whilst our assays do not measure substrate turnover due to microbial growth (NaN_3 and/or short incubation time) some diffusion of substrate into metabolically-active yet genetically-latent or -moribund cells can occur[16].

e) Irradiation may produce or release organic molecules which inhibit some enzymes.

It was noticeable that all 4 enzyme controls (no substrate) increased with irradiation; most dramatically in the moist soils (figure 3). Apparent increases in arylsulphatase and phosphatase activities can be accounted for by the solubilization of humic materials[18] which, in turn, give artificially-high readings during the spectrophotometric determination of p-nitrophenol.

With the β-1,3 glucanase controls it is probable that irradiation releases glucose from sugar polymers (in both humic matter and microbial biomass) and that this phenomenon becomes more pronounced with increasing dosages. In addition, the indigenous substrate level may increase due to cell lysis.

In the case of urease some irradiation-induced deamination of humic matter may occur[19] especially fulvic acids[20]. Proteins and nucleic acids from lysed cells[21,22] may be mineralized whilst subsequent oxidation of ammonia will be restricted due to the death of the nitrifiers. All these factors will tend to increase the ammonium levels in the controls. Less likely (because of our use of K_2CO_3 at the completion of the assay period[9]) is the exchange of adsorbed NH_4^+ for H^+ produced by ionization of water. A change in the accessibility of the enzymes may also occur if irradiation alters the humic material with which they are associated.

Incidentially γ-irradiation is often presented as a less harsh method of sterilizing soil than autoclaving. This may well be so at low radiation levels but out soils which received 20 and 50 Mrad showed a considerable decline in aggregate size – possibly due to the depolymerization of the organic materials which hold the primary particles together[23] – and a noticeable hydrophobic tendency.

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