RESEARCH REPORT

SYNTHESIS OF USEFUL ORGANIC COMPOUNDS USING COMMERCIAL HYDROLYTIC ENZYMES

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ABSTRACT

Enzyme catalysis is most attractive for the synthesis and modification of biologically relevant classes of organic compounds which are difficult to prepare and to handle by conventional means.

In this study, commercial hydrolytic enzymes were used in the preparation of two novel organic compounds with excellent properties and application as fragrance and flavour – isoamyl acetate, a substance with peculiar banana fragrance and (-)-menthyl butyrate, a substance with cooling and refreshing minty effects.

In the synthesis of isoamyl acetate, reaction consisting of acetic anhydride and isoamyl alcohol were catalysed using lipase from *Rhizomucor miehei* (Lipozyme IM-20), based on the method by Krishna *et al.*, (2001). Yields above 93 % were achieved with substrate concentration as high as 1.5 M and more than 150 g/L isoamyl acetate concentrations were obtained employing relatively low enzyme concentration of 10 g/L after 100 hours of incubation. The product was then subjected to characterization using Fourier-transform infra-red (FT-IR) spectrophotometer, gas chromatography-mass spectrometer (GC-MS) and nucleus magnetic resonance (NMR) to ensure purity. Biological activity assay of the compound was done for their cytotoxic effect against HL60 (human promyelocytic leukemia) and breast cancer (MCF7) cell lines. In the actimicrobial activity assay, microbes tested were MRSA (*Methicillin resistant Staphylococcus aureus*), *Bacillus substilis* and *Pseudomonas aeruginosa*. Isoamyl acetate was found biologically inactive towards targeted microbes and both cancer cell lines.

In this synthesis of (-)-menthyl butyrate, among seven lipases tested, *Candida rugosa* lipase exhibited the best ability to catalyze the resolution of (±)-menthol in organic solvent. To ensure purity of the compound, it was characterized using Fourier-transform infra-red (FT-IR) spectrophotometer, gas chromatography (GC), thin layer chromatography (TLC) and gas chromatography-mass spectrometer (GC-MS).

ABSTRAK

Pemangkinan berenzim amat menarik terutamanya dalam sintesis dan modifikasi sebatian organik berasaskan biologi yang biasanya sukar untuk disediakan serta dikendalikan melalui kaedah konvensional.

Dalam kajian ini, enzim hidrolitik komersial digunakan dalam penyediaan dua kompaun organik unggul dengan cirri-ciri dan kegunaan yang menarik sebagai bauan dan perisa – isoamil asetat, sejenis sebatian dengan bauan pisang yang menarik dan (-)-menthil butirat, sejenis sebatian dengan kesan-kesan penyejukan yang menyegarkan.

Dalam sintesis isoamil asetat, bahan tidakbalas yang mengandungi asid asetik dan alkohol isoamil telah dimangkinkan menggunakan lipase daripada *Rhizomucor miehei* (Lipozyme IM-20), berdasarkan kepada kaedah oleh Krishna *et al.* (2001). Hasil melebihi 93 % telah diperolehi menggunakan kepekatan substrat setinggi 1.5 M dan lebih daripada 150 g/L kepekatan isoamil asetat telah diperolehi menggunakan kepekatan enzim yang rendah iaitu 10 g/L, selepas 100 jam tempoh pengeraman. Pencirian ke atas produk tersebut dijalankan menggunakan spektrofotometer lembayung merah Fourier-transform (FT-IR), spektrometer jisim-kromatografi gas (GC-MS) dan Resonans Magnetik Nukleus (NMR) untuk penentuan ketulenan. Ujian aktiviti biologi kompaun telah dijalankan untuk menguji kesan cyto-toksik kompaun terhadap jujukan sel-sel HL60 (leukemia promyelocytic manusia) dan kanser payudara (MCF7). Dalam ujian aktiviti antimikrobiol, mikrob-mikrob yang diuji adalah MRSA (*Staphylococcus aureus* tahan *Methicillin*), *Bacillus substilis* dan *Pseudomonas aeruginosa*. Isoamil asetat didapati tidak aktif-biologi terhadap mikrob-mikrob sasaran dan kedua-dua jujukan sel-sel kanser.

Dalam sintesis (-)-menthil butirat, antara tujuh lipase yang diuji, lipase *Candida rugosa* mempamerkan keupayaan terbaik untuk memangkinkan resolusi (±)-menthol dalam pelarut organik. Untuk memastikan ketulenan kompaun, ia telah dicirikan menggunakan spektrofotometer Infra-merah Fourier-transform (FT-IR), kromatografi gas (GC), kromatografi lapisan tipis (TLC) dan spektrometer jisim-kromatografi gas (GC-MS).

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