University of Canterbury

Mid Year Examination and Test Period 2009

Prescription Number(s):	CHEM 325 BCHM 302
Paper Title:	Biological Chemistry

Time Allowed:	2.5 hours
Number of pages:	TEN plus two separate answer sheets

This paper is in **THREE** sections.

Sections A and B are worth 30 marks each. Section C is worth 60 marks

Section A: Answer **THREE** questions. All questions in this section are of equal value

Section B: Answer **ONE** question from this section.

Section C: Answer **BOTH** questions.

TURN OVER

SECTION A

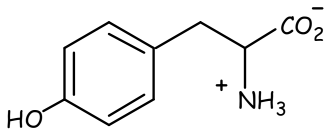
Answer **THREE** questions. All questions in this section are of equal value.

1. (a) Transamination and decarboxylation are commonly encountered reactions in secondary metabolism. What product is formed when tyrosine undergoes:

(i) decarboxylation?

(ii) transamination?

(iii) transamination followed by decarboxylation?



tyrosine

(b) In the biosynthesis of natural products of polyketide origin:

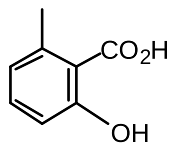
(i) What is the usual “starter” unit?

(ii) Give the structures of two alternative starter units.

(iii) What is the usual “repeat” unit?

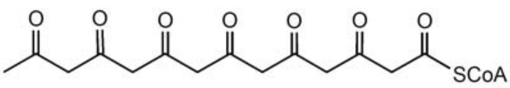
(iv) Give the structure of an alternative repeat unit.

(v) In polyketide biosynthesis it is often observed that an oxygen atom is "missing". An example would 5-methylsalicylic acid. In the biosynthesis of 5-methylsalicylic acid how is the oxygen "lost"?

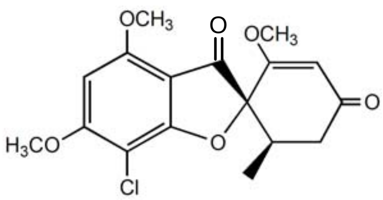


5-methylsalicylic acid

2. Griseofulvin, a compound of polyketide origin, is formed from a heptaketide. This polyketide chain can be folded in more than one way to produce griseofulvin. Draw out two **alternative** folding patterns of the polyketide chain that would fit the observed functionality of griseofulvin.

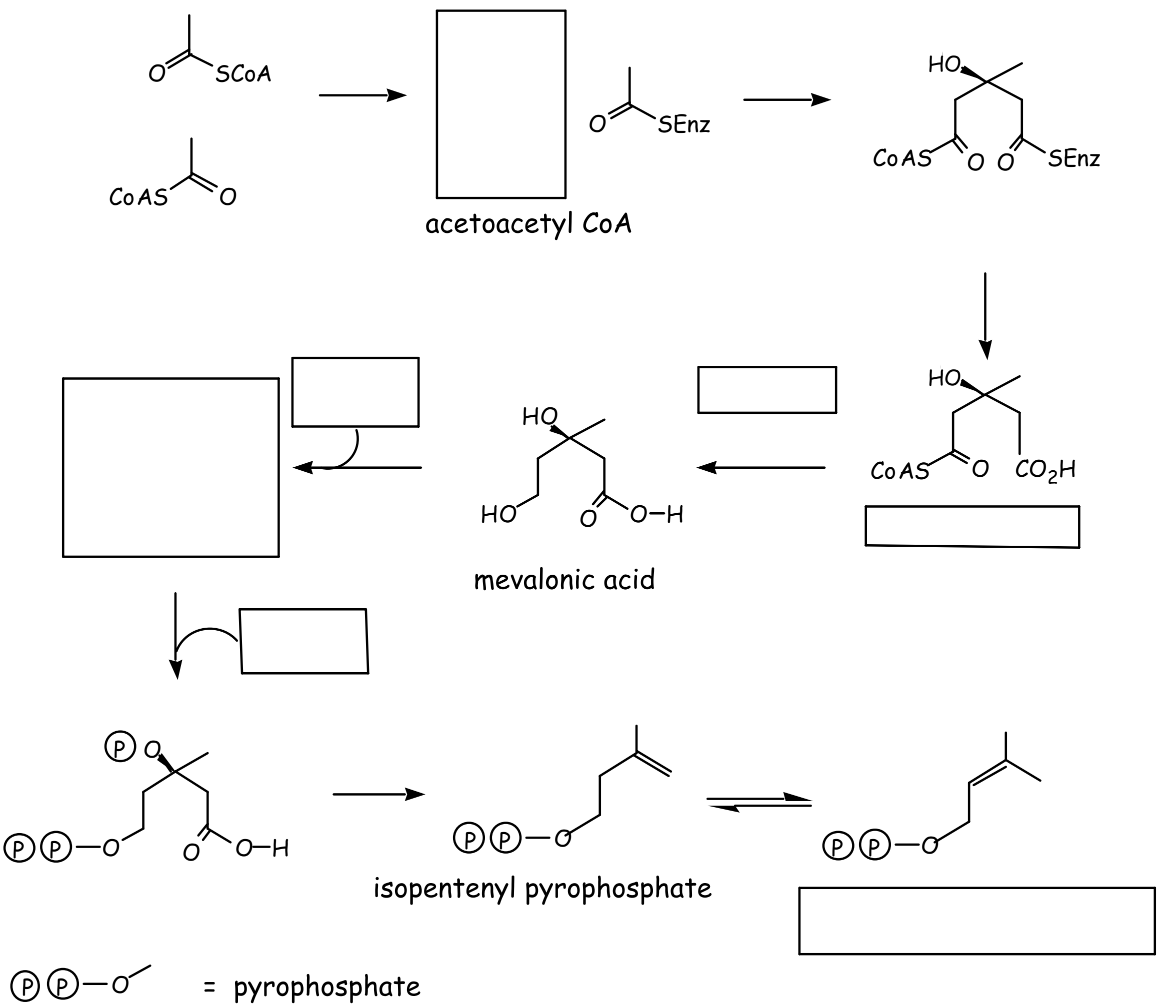


Heptaketide unit

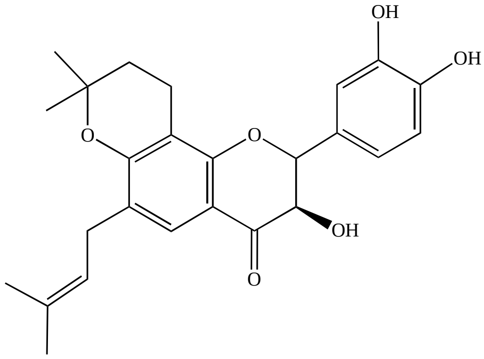


Griseofulvin

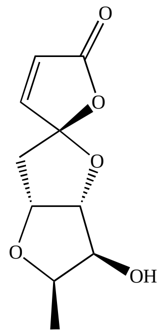
3. The initial steps in the formation of compounds of ISOPRENOID origin by the MEVALONATE pathway is shown in outline in the diagram below. On the accompanying sheet fill in the missing information on structures, names and reactants in the boxes provided. Show details of mechanisms where possible. (Tie your answer to your exam booklet)



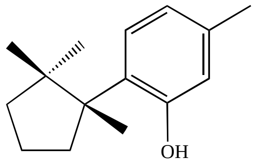
4. What are the biosynthetic origins of **TWO** of the three following compounds (A, B and C)? Note that some compounds could be of "mixed" biosynthetic origin. For each compound indicate (delineate) the "building blocks" of each structure (*ie* the isoprene units, the polyketide chain, the starter unit, the repeat units, SAM, loss of O, amino acid *etc*.). Fill in your answer on the accompanying sheet and tie your answer to your exam booklet.



A

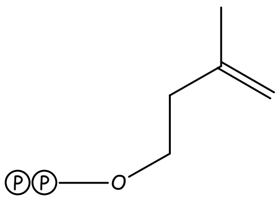


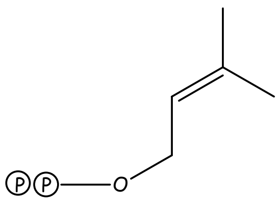
B



C

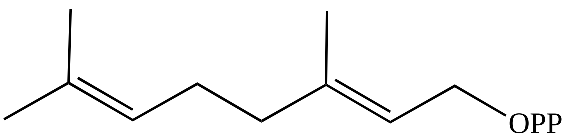
5. The C5 isoprenoid intermediates (below) are comparable in structure, but differ markedly in reactivity and the role that each plays in isoprenoid biosynthesis.





(a) Explain the difference in reactivity.

(b) Illustrate the role each compound plays by outlining the formation of geranyl pyrophosphate from the two C5 isoprenoid intermediates.



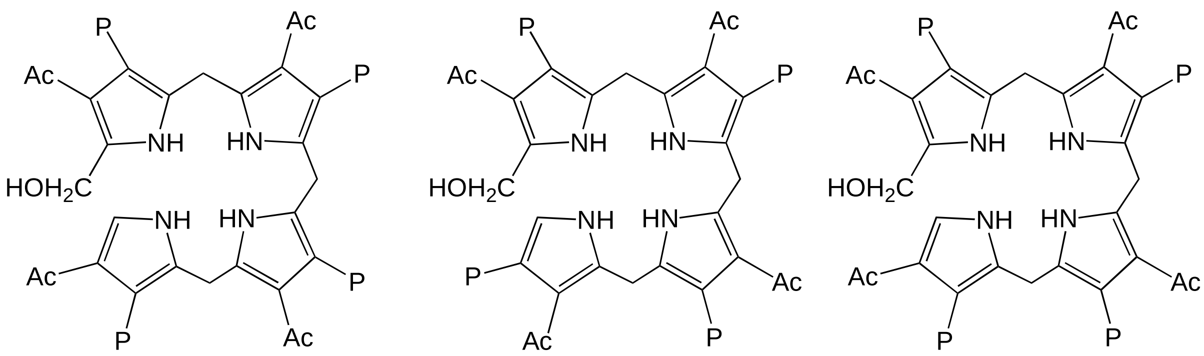
Geranyl pyrophosphate

SECTION B

Answer **EITHER** Question 6 **OR** Question 7 from this section.

6. (a) The three structures below are isomers of 1-hydroxymethylbilane, a labile intermediate in tetrapyrrole biosynthesis that spontaneously cyclizes in solution in the absence of the enzyme uroporphyrinogen III synthase (UROS). The substituents on the ring are acetate (Ac) and propionate (P).

Which is the correct biological isomer? Sketch the products that would be formed after incubation of each of the compounds with UROS.



1. 2. 3.

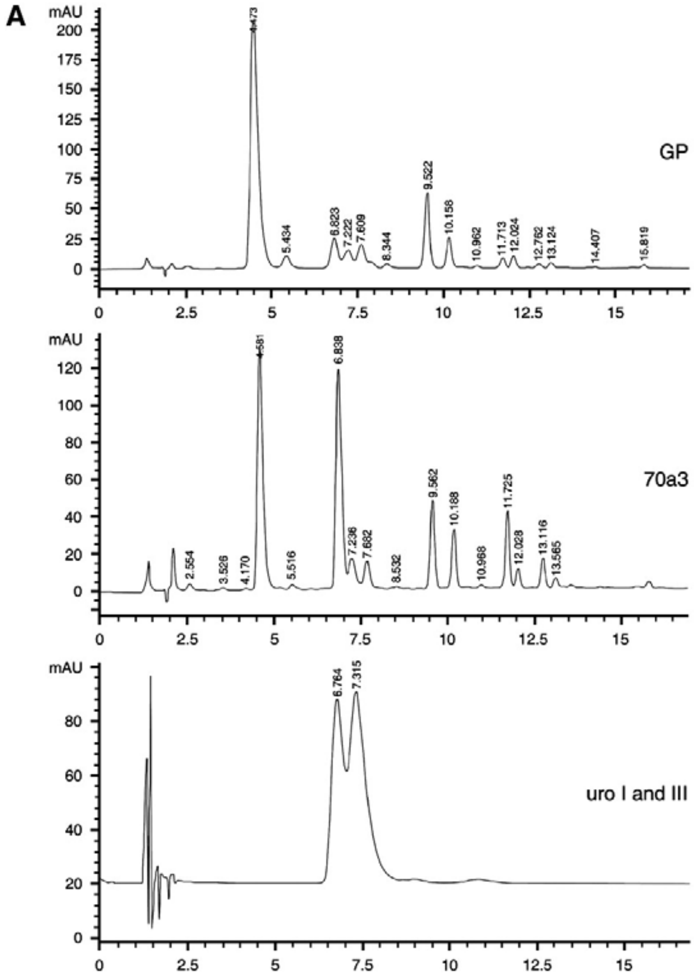
(b) Outline briefly the enzymic steps that take place to convert uroporphyrinogen III to haem.

(c) A line of barley (70a3) exhibited severe necrotic lesions (*i.e.* regions of dead tissue) on its leaves, indicating phototoxic damage had occurred. An extract of the leaves was analysed by HPLC and compared to normal barley plants (GP). The HPLC trace (shown on the following page) shows the results of this analysis together with the trace for standards of purified uroporphyrin I and uroporphyrin III (uro I and uro III). Briefly explain what the HPLC data demonstrate. Can this explain the phenotype seen in line 70a3?

Question 6 continued on following page

Question 6 continued

The traces show the times for the major peaks present in extracts of normal barley (GP) and a mutant line that exhibited necrotic lesions on the leaves. In the lower panel, two peaks are seen for uro I at 6.7 min, and uro III at 7.3 min.
(Data from Ayliffe et al. (2009) Plant Cell 21:814-831)



Time of elution from HPLC column (min)

7. In spearmint (*Mentha spicata* L.) the principal component of the essential oil is (–)-carvone, whereas peppermint (*Mentha* x *piperita* L.) contains almost exclusively (–)-menthol. This difference is due to the presence of either limonene-3-hydroxylase (enzyme **PM** in Fig. 3), or limonene-6-hydroxylase (enzyme **SM**) in the two species.

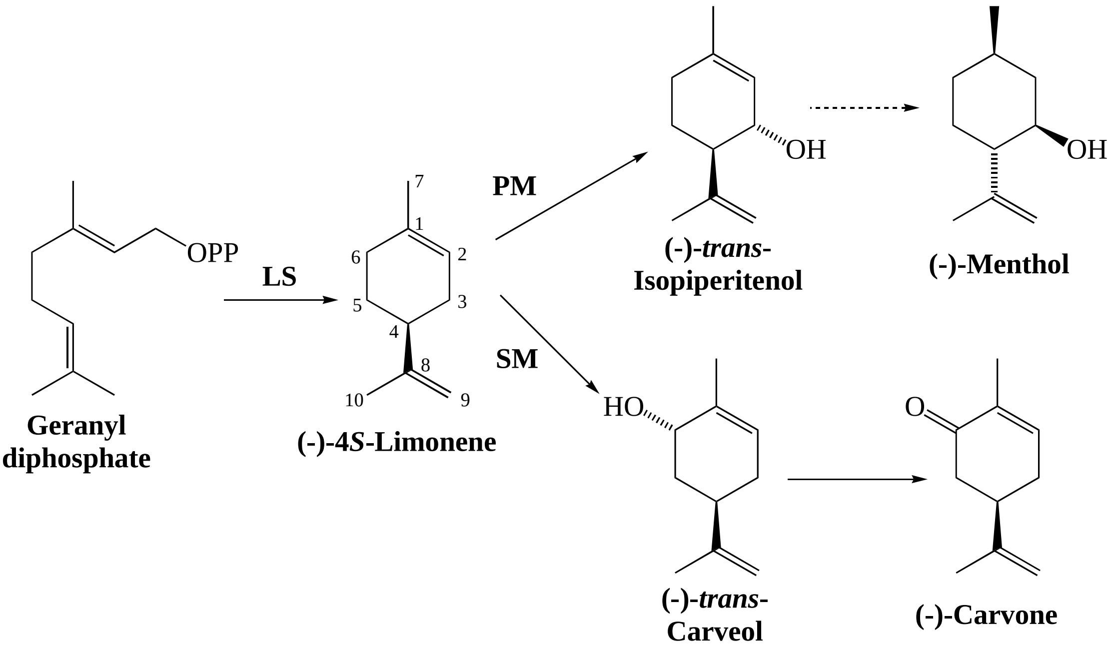


Fig. 3 Biosynthetic pathways for menthol in peppermint, and carvone in spearmint. Menthol is made from *trans*-isopiperitenol by a further 5 enzymic steps (shown as dotted line), whereas a single dehydrogenase step gives rise to carvone from *trans*-carveol. **LS** = limonene synthase; **PM** = limonene 3-hydroxylase; **SM** = limonene-6-hydroxylase.

(a) Limonene is synthesised from the C10 biosynthetic intermediate geranyl diphosphate (also called geranyl pyrophosphate, GPP) by limonene synthase, shown as **LS** in Figure 3. Like many of the enzymes of isoprenoid biosynthesis, the reaction is thought to proceed via a carbocation intermediate. Draw out a possible reaction mechanism for **LS**.

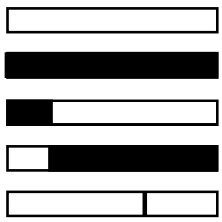
(b) What likely class of enzyme are **PM** and **SM**, and therefore what cofactor(s) will they require? How might these two enzymes have evolved in the different mint species?

Question 7 continued on following page

Question 7 continued

(c) Genes for the two enzymes, **SM** and **PM**, have been identified and sequenced, and the proteins they encode are 70% identical at the amino acid level. The enzyme from spearmint (**SM**) catalyses exclusively the incorporation of a hydroxyl group at C6, whereas that from peppermint (**PM**) catalyses exclusively the incorporation at C3 (Table 1 below). To identify residues in the proteins that were responsible for the specificity, several mutant or chimeric proteins were generated and their activity for hydroxylation at C3 and C6 were tested (Table 1).

Table 1 Activity of limonene hydroxylase from spearmint (**SM**) and peppermint (**PM**) for hydroxylation at either C6 or C3 position of limonene. As well as the wild-type enzymes, the data for 2 chimeric constructs are given in which *N*-terminal domains from **SM** were fused to *C*-terminal domains of **PM**, and *vice versa*. In addition, the data for one point mutant of the spearmint enzyme, **SM**-F363I is shown. This mutation changes a conserved residue in limonene 6-hydroxylase to the equivalent conserved residue in limonene 3-hydroxylase. *[Data from Schalk & Croteau (2000) PNAS 97: 11948-11953]*.



	Limonene hydroxylase activity (relative units)	
	C3	C6
SM wild-type	0	47
PM wild-type	100	0
Chimera 1	0	0.46
Chimera 2	4.7	0
SM-F363I	100	0

Discuss the implications of the data in Table 1 for the regions of limonene hydroxylase that affect the activity and regiochemical specificity of these enzymes.

SECTION C

Answer **BOTH** questions from this section.

8. Discuss the importance of water as a ligand in biological systems. Your answer should refer explicitly to:

   (i) the chemistry of sodium and potassium ions;

   (ii) the effectiveness of Magnetic Resonance Imaging contrast agents;

   (iii) possible mechanisms of hydrolytic metalloenzymes.

9. Electron transport and oxygen binding are fundamental components of respiration.

   (a) Explain why metal ions are used in these components.

   (b) What features of metal complexes are typically associated with fast electron transfer reactions?

   (c) Discuss whether or not the features you describe in (b) are relevant to biological systems.

END OF PAPER

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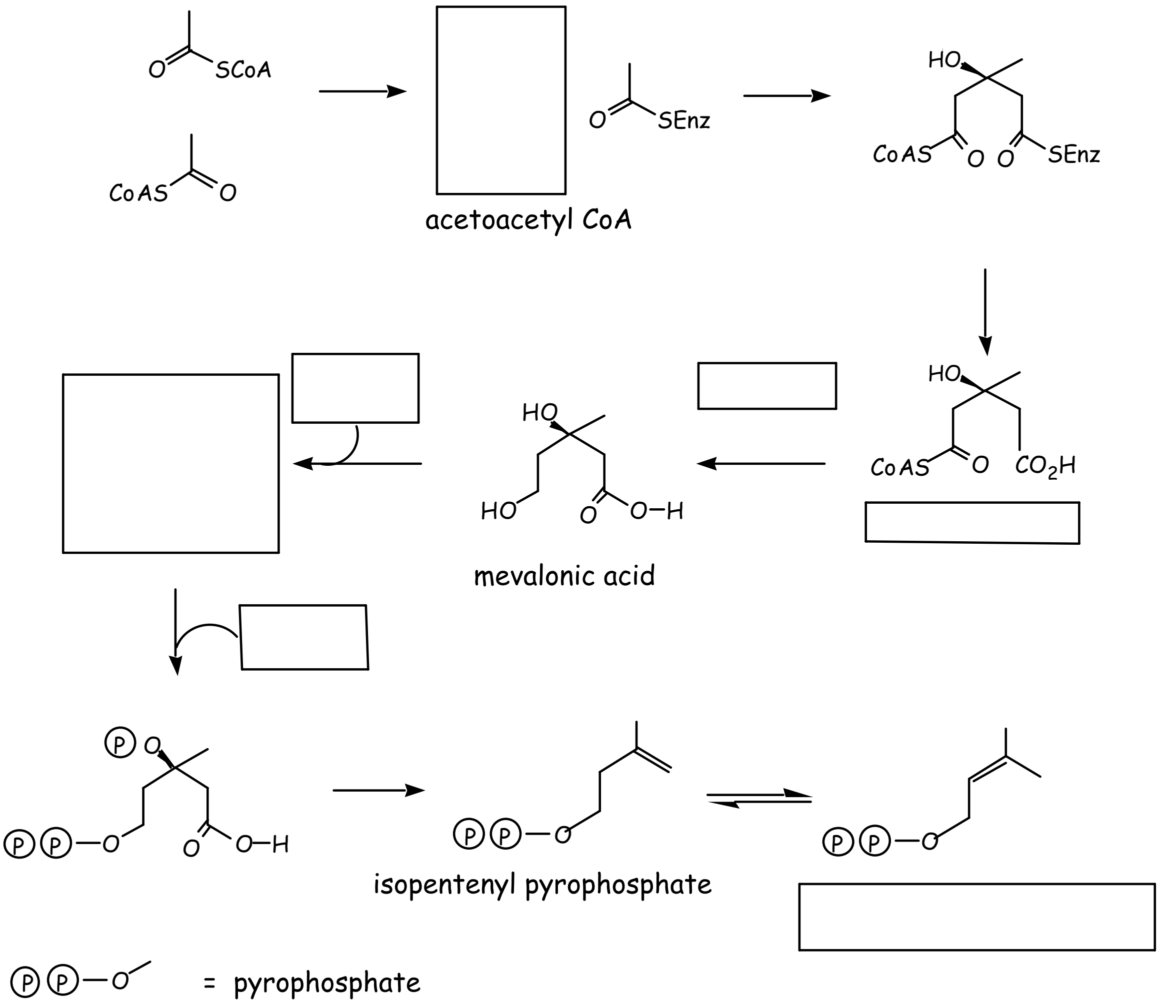
Question 3. **Fill in the missing information on structures, names and reactants in the boxes provided. Show details where possible. Attach this sheet to your examination booklet**



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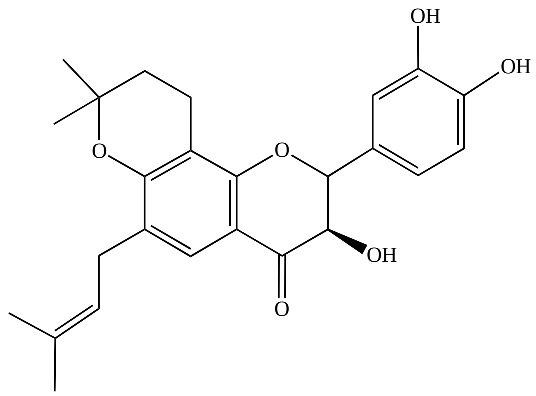
Question 4. Analyse structures and attach this sheet to your examination booklet

A



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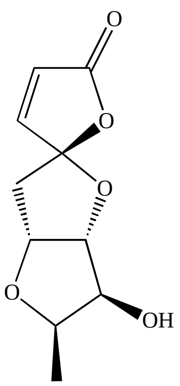

C