Phytochemistry, Vol. 21, No. 3, pp. 559–563, 1982.
Printed in Great Britain.

0031-9422/82/030559-05$03.00/0

EFFECTS OF METAL IONS ON BENZYLGLUCOSINOLATE DEGRADATION IN *LEPIDIUM SATIVUM* SEED AUTOLYSATES

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(*Revised received* 5 *August* 1981)

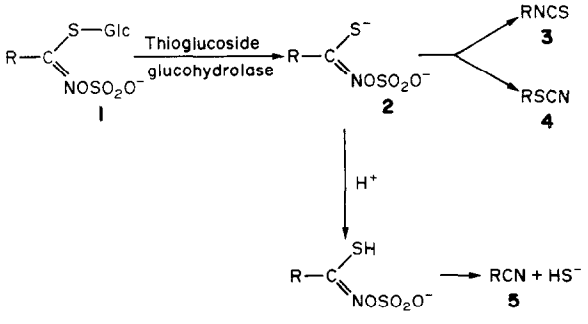
Key Word Index—*Lepidium sativum*; Cruciferae; glucosinolate degradation.

Abstract—The effects of varying concentrations of Fe^{2+} (5×10^{-5}–5×10^{-1} M) on benzylglucosinolate degradation in *Lepidium sativum* seed autolysates were investigated. Increased glucosinolate decomposition was observed over the whole range with a maximum effect at *ca* 6×10^{-3} M Fe^{2+}, at which point glucosinolate degradation was more than three times that obtained in the absence of added Fe^{2+}. Nitrile formation was especially enhanced in the presence of all concentrations of Fe^{2+} studied, and maximum amounts were obtained at *ca* 6×10^{-3} M Fe^{2+}, when a more than four-fold increase over quantities produced in the absence of Fe^{2+} was observed. Thiocyanate formation was also promoted with a maximum at *ca* 4×10^{-3} M Fe^{2+}, but isothiocyanate production was considerably reduced in all cases. It is suggested that Fe^{2+} inhibits isothiocyanate formation by interfering with the availability of ascorbic acid which is a proven co-factor for most thioglucosidase isoenzymes, but that an Fe^{2+}-ascorbate complex might then be responsible for promoting enzymic production of nitrile. The effects of a limited range of concentrations of Fe^{3+} and Cu^{+} were also studied, and results related to those for Fe^{2+}. The relevance of the findings to natural systems and to glucosinolate-containing foods is briefly discussed.

INTRODUCTION

Glucosinolates (**1**) are readily decomposed enzymically to give three main types of products, isothiocyanates, thiocyanates and nitriles, with glucose and sulphate as by-products. The basic theory of glucosinolate degradation, as shown in Scheme 1, postulates that a thioglucosidase first generates an aglucone (**2**) which undergoes a spontaneous chemical decomposition to form an isothiocyanate (**3**) by a Lossen rearrangement and a nitrile (**3**) by a mechanism which supposedly is totally dependent on the pH of the system. The formation of thiocyanate (**4**) is not completely understood, but the existence of a discrete thiocyanate-forming factor or enzyme has recently been proved [1].

Scheme 1 was originally derived mainly from model system studies but it does not adequately explain all observations with regard to the formation of the three main products in many natural systems.



Scheme 1. Enzymic degradation of glucosinolates.

A major problem concerns the mechanism of nitrile production. There is ample evidence in support of the proton-dependent mechanism based mainly on investigations using pure aglucones or pure glucosinolates plus thioglucosidase preparations [2, 3], and although the mechanism may apply in some natural systems, there is also considerable evidence that it cannot apply in all cases. Thus many workers have demonstrated the formation of large amounts of nitrile *in vivo* at pH levels at which the protonation mechanism cannot be operative (i.e. at pH 7 or above), and frequently in these cases nitrile has even been the major glucosinolate product [4–7]. Therefore, there must be some alternative mechanism of nitrile formation. However, this mechanism is not dependent on the identity of the glucosinolate since allylglucosinolate gives mainly isothiocyanate in *Brassica oleracea* autolysates [8] and mainly nitrile in *Farsetia aegyptia* autolysates (at pH 7) [6]. In the latter plant system there must be some quality or factor which directs glucosinolate degradation to nitrile via the alternative, non-proton-dependent mechanism. The presence or absence of such a factor would also explain the observation [9] that some plant systems inherently are mainly isothiocyanate forming (e.g. *Brassica oleracea* seeds [8], *Nasturtium officinale* seeds and leaves [9], and *Barbarea praecox* seeds [9]), whilst others are naturally mainly nitrile forming (e.g. *Lepidium sativum* seeds [7] and leaves [10], and leaves of some *Farsetia* species [6]). The influence of pH could not have been significant in these particular *in vivo* studies.

It has been suggested that the unknown factor may be the metal ion content of the system [3, 11], since it

has been known for many years from model system studies that nitrile formation can be promoted by metal ions [2, 12–19]. Austin and Gent were able to convert 2 - hydroxybut - 3 - enylglucosinolate itself in the absence of any enzyme into the corresponding nitrile using an 8 M excess of Fe^{2+} [12]. Similarly, Youngs and Perlin showed the catalytic effect of Fe^{2+} in converting allylglucosinolate to nitrile [13]. Although these non-enzymic processes are important, there is no evidence that they have any direct bearing on the natural system. However, other model system work has also included an enzyme preparation or has been based on the aglucone, which is the enzymically produced species (**2**, Scheme 1). Thus Miller observed that Fe^{2+} decomposed the aglucone of allylglucosinolate into the nitrile [2]. Austin *et al.* [14] found that a *Sinapis alba* enzyme preparation in the presence of added Fe^{2+} converted isolated 2-hydroxybut - 3 - enylglucosinolate predominantly into the nitrile product, but in the absence of Fe^{2+} the main product was 5 - vinyloxazolidine - 2 - thione (goitrin, the cyclized product formed spontaneously from 2 - hydroxybut - 3 - enyl isothiocyanate). Similar results have also been obtained with this glucosinolate using a *Crambe abyssinica* enzyme preparation [15, 16]. The majority of these studies have been carried out using Fe^{2+}, and it has been claimed that the observed phenomenon of induced nitrile formation is specific for this ion, since others, such as Fe^{3+}, Co^{2+}, Mn^{2+} and Sn^{2+}, were ineffective, whilst Cu^{2+} inhibited enzymic action [15]. A direct reducing effect of Fe^{2+} has been eliminated [15] and only catalytic amounts were necessary when enzyme was also included in the model systems [15, 16].

To the best of our knowledge the effect of added metal ions on genuine *in vivo* glucosinolate decomposition has received no attention, with the exception of a brief comment that the glucosinolates of *Brassica campestris* seed meal degraded to yield nitriles in the presence of certain metallic salts, notably ferrous sulphate (Youngs, C. G., unpublished). Therefore, such an *in vivo* survey would be valuable, so that the results may be compared with the wealth of consistent data for model systems. Since *L. sativum* has been extensively studied in this laboratory this system was employed to conduct the present detailed *in vivo* survey of the effects of Fe^{2+} on glucosinolate degradation, with the main objective of evaluating the implication of metal ions in an alternative (i.e. non-proton-dependent) *in vivo* mechanism of nitrile production. Nitriles can also be generated from glucosinolates in other ways, e.g. non-thioglucosidase-induced mechanisms [1, 20–22] and non-enzymic, high temperature thermal degradation [23], but neither of these processes is directly relevant to this investigation based on *in vivo* autolysis at ambient temperatures.

RESULTS AND DISCUSSION

The major glucosinolate of *Lepidium sativum* is benzylglucosinolate, and others are only present in trace amounts [7]. Benzyl cyanide, isothiocyanate and thiocyanate, together with trace amounts of benzyl alcohol and benzaldehyde, were readily identified in *L. sativum* seed autolysates by means of GC and GC/MS. The detection of the alcohol and aldehyde is further evidence in support of their glucosinolate origin [7], but since they were produced in very small quantities (too small for accurate measurement), consideration here is limited to the three main products.

Table 1 gives results for the amounts of benzylglucosinolate degradation products obtained in *L. sativum* seed autolysates in the presence of varying concentrations of added Fe^{2+}. Considering first the extent of glucosinolate degradation, it can be seen that Fe^{2+} exerted a considerable 'catalytic' effect over the whole concentration range studied (5×10^{-5}–5×10^{-1} M Fe^{2+}), in that in all cases more total products were formed than in the absence of added Fe^{2+}. Although the word 'catalytic' is used, we present no direct evidence that the phenomenon is one of genuine catalysis (indeed some findings are against this), but the term is used in a generalized context to facilitate discussion. Furthermore, the results show conclusively that Fe^{2+} considerably promoted nitrile formation *in vivo*, and again over the whole range of concentrations studied. Thus, taken in conjunction with the previously mentioned results from model system studies, these findings support the proposal for an alternative, non-proton-dependent mechanism of nitrile production from glucosinolates which is Fe^{2+}-catalysed. This mechanism could explain the previously mentioned 'anomalies' with regard to natural systems, in that natural variation in endogeneous Fe^{2+} would account for varying extents of nitrile generation in different systems.

From Table 1 it can be seen that the catalytic effect of Fe^{2+} was most pronounced with regard to nitrile production, since in all instances the extent of the increase in nitrile concentration over its concentration in the absence of added Fe^{2+} was far greater than for any other product. However, Fe^{2+} also promoted thiocyanate formation, except at relatively high concentrations (i.e. greater than *ca* 4×10^{-2} M). This is the first report of a metal ion catalysing thiocyanate formation *in vivo*. This finding may be of subsequent value in attempts to elucidate the mechanism of glucosinolate catabolism to thiocyanate. In contrast, Fe^{2+} was an effective inhibitor of isothiocyanate formation and over the whole range of concentrations employed far less isothiocyanate was obtained in the presence of Fe^{2+} than in its absence (from less than 50% to less than 4%).

Maximum glucosinolate degradation was observed at a concentration of *ca* 6×10^{-3} M Fe^{2+}, at which point over three times as much glucosinolate was decomposed compared with the blank determination in the absence of added Fe^{2+}. A similar maximum in nitrile formation was obtained at exactly the same level, and corresponded to a greater than four-fold increase in nitrile over the blank. Clearly for this particular *in vivo* system, this represents the optimum catalyst concentration for accomplishing maximum glucosinolate degradation to nitrile. It is interesting that a similar maximum was also observed for thiocyanate production, although this was at a very slightly lower concentration of Fe^{2+} (4×10^{-3} M). In these conditions more than double the amount of thiocyanate was produced compared with the blank determination. Since the maxima for nitrile and thiocyanate formation are so similar, and since the plot-

Table 1. Amounts of benzylglucosinolate degradation products formed in *Lepidium sativum* seed (1.0 g) autolysates in the presence of varying concentrations of Fe^{2+}

Fe^{2+} Concentration (M)	Benzyl cyanide		Benzyl thiocyanate		Benzyl isothiocyanate		Total products (mg)
	mg	%	mg	%	mg	%	
0(Blank)	2.99	67.2	0.70	15.8	0.76	17.0	4.45
5×10^{-5}	5.67	77.8	1.26	17.4	0.35	4.8	7.28
1×10^{-4}	6.40	79.4	1.40	17.3	0.26	3.3	8.06
5×10^{-4}	8.06	83.9	1.43	14.9	0.12	1.2	9.61
8×10^{-4}	8.22	84.4	1.42	14.6	0.10	1.0	9.74
9×10^{-4}	8.67	84.9	1.45	14.3	0.09	0.8	10.21
1×10^{-3}	8.72	84.8	1.48	14.4	0.08	0.8	10.28
2×10^{-3}	9.37	85.0	1.57	14.3	0.08	0.7	11.02
3×10^{-3}	9.84	85.1	1.65	14.2	0.08	0.7	11.57
4×10^{-3}	10.43	85.1	1.76	14.3	0.07	0.6	12.26
5×10^{-3}	10.84	86.6	1.60	12.8	0.07	0.6	12.51
6×10^{-3}	12.88	89.6	1.42	9.9	0.07	0.5	14.37
7×10^{-3}	12.03	89.9	1.27	9.6	0.07	0.5	13.37
8×10^{-3}	11.24	89.6	1.24	9.9	0.07	0.5	12.55
9×10^{-3}	10.88	89.4	1.23	10.1	0.06	0.5	12.17
1×10^{-2}	10.43	89.8	1.13	9.7	0.06	0.5	11.62
2×10^{-2}	9.80	91.0	0.92	8.5	0.05	0.5	10.77
5×10^{-2}	9.52	93.5	0.63	6.2	0.03	0.3	10.18
1×10^{-1}	9.14	93.8	0.57	5.9	0.03	0.3	9.74
5×10^{-1}	7.32	96.5	0.24	3.2	0.03	0.3	7.59

ted data give curves with very similar shapes, this suggests that the catalytic mechanism might be the same for both products. Again this deduction might be of assistance in consideration of the processes of thiocyanate formation. The mechanism of *in vivo* production of isothiocyanate in the presence of Fe^{2+} is clearly entirely different, in that no maximum of formation was attained and concentration decreased regularly with increasing Fe^{2+} concentration.

Considering the percentage figures given in Table 1, it is interesting to note that no maximum in nitrile production is apparent, and a regular increase is observed with increasing Fe^{2+} concentration to the point at which it is produced in nearly quantitative yield. Conversely, isothiocyanate and thiocyanate both show fairly regular decreases in concentration with increasing Fe^{2+}. Therefore, if the overall process of glucosinolate degradation to the various products is regarded as a competitive system, then Fe^{2+} promotes the non-proton-dependent mechanism to yield nitrile at the expense of the 'normal' degradative routes to isothiocyanate and thiocyanate.

Although all the above findings display a convincing regularity of behaviour and are more meaningful to natural systems than are model system studies, they are more difficult to explain and rationalize. However, the data do show that there are two main mechanisms of *in vivo* glucosinolate degradation in the presence of Fe^{2+}, one that is catalysed by Fe^{2+} to yield excess nitrile (and probably thiocyanate also), and the other that is inhibited by Fe^{2+} giving reduced amounts of isothiocyanate. The obvious explanation of the latter behaviour is that the presence of the metal ion somehow interferes with the thioglucosidase enzyme system, and hence isothiocyanate production is suppressed with increasing concentration of inhibitor, as shown by the data in Table 1. The problem is to explain the much enhanced production of nitrile (and thiocyanate) under the same circumstances, and also the maximum of activity at 6×10^{-3} M Fe^{2+}.

One possibility for increased nitrile production is non-enzymic formation from the glucosinolate itself, and as already mentioned there is precedence for this on the basis of model system studies [12, 13]. However, in these cases large amounts of Fe^{2+} were generally necessary, although Youngs and Perlin identified nitrile as a product from allylglucosinolate using only an equimolar quantity of Fe^{2+} [13]. In addition they identified bis(β-D-glucopyranosyl) sulphide in the reaction mixture rather than glucose (as is normally obtained) or 1 - thio-D-glucose [13]. To explain this interesting observation they suggested a convincing mechanism of metal ion catalysed glucosinolate degradation to nitrile based on the formation of an intermediate glucosinolate–metal ion complex, for which there was some precedence [24], which comprised of two molecules of glucosinolate to one Fe^{2+} [13]. Our results do not, however, support the formation of such a complex *in vivo* for the following reason. *L. sativum* seeds employed in this work contained at least 53 mg/g benzylglucosinolate (calculated on a fr. wt basis from maximum levels in Table 1) which represents *ca* 0.12 M, whilst only *ca* 6×10^{-3} M added Fe^{2+} was required for maximum degradation to nitrile. This is a ratio of *ca* 20:1 rather than 2:1, and hence there was insufficient Fe^{2+} added to the *in vivo* system to form optimum amounts of such a complex at the point of maximum degradation (the amount of endogeneous Fe^{2+} is so little as not to affect significantly this crude calculation). In other instances even larger excesses of Fe^{2+} were required

to effect non-enzymic glucosinolate degradation to nitrile, and the mechanism was basically reductive [12].

Therefore, since the optimum concentration of Fe^{2+} for nitrile formation in *L. sativum* autolysates was insufficient for the known non-enzymic processes, the only other reasonable possibility is that the presence of Fe^{2+} somehow affected the course of the enzyme mechanisms of glucosinolate degradation. This could involve either the thioglucosidase itself or ascorbic acid, which is a proven co-factor for most thioglucosidase isoenzymes. In this work it is more likely that Fe^{2+} interfered with the availability of ascorbate for the following reasons. First, it has been shown specifically with benzylglucosinolate and an *L. sativum* thioglucosidase preparation that ascorbate did genuinely promote isothiocyanate production for that system, and that in its absence isothiocyanate formation was much decreased [25]. It was also shown with the same system that thiocyanate formation was suppressed by ascorbate but that ascorbate was not necessarily involved in enzymic degradation to nitrile since in its absence there was a smooth increase in nitrile concentration with time but in its presence formation was erratic [25]. So a lesser availability of ascorbate would not be expected to inhibit the generation of these two products in the same manner. Thus it could be that in the *in vivo* system an Fe^{2+}–ascorbate complex was formed which suppressed isothiocyanate formation by removing available free ascorbate from the system, but which itself acted as a modified and improved co-factor to promote increased enzymic glucosinolate degradation to nitrile. Such behaviour would not be contrary to modern concepts of the mode of action of thioglucosidase and ascorbate in glucosinolate degradation [26, 27].

Whatever the cause of increased nitrile production from glucosinolates in the presence of added Fe^{2+}, the observed maximum in activity at *ca* 6×10^{-3} M Fe^{2+} is a particularly interesting feature, but on present evidence no explanation of this can be offered. There seems to be no direct correlation with the concentrations of any of the reactants in the system (benzylglucosinolate, *ca* 0.12 M; ascorbic acid, *ca* 3.4×10^{-6} M [28]; and endogeneous total iron, *ca* 4×10^{-5} M [15]). Such a maximum has not been observed before, although previous workers have not evaluated such a wide concentration range. For example, data reported by Tookey and Wolff extend only from 1×10^{-4} to 1.24×10^{-3} M Fe^{2+} [15], and clearly if their system showed a maximum and if its value was about the same as that observed in our work, then their curve did not extend to sufficiently concentrated Fe^{2+} levels.

A limited series of experiments was also carried out using Fe^{3+} and Cu^{+} as the metal ion catalysts, and the results are given in Table 2. The range of concentrations studied, which was chosen to cover the peak of activity shown by Fe^{2+}, is too limited for many definite conclusions, but the following observations are relevant. Both Fe^{3+} and Cu^{+} clearly affect the course of *in vivo* glucosinolate degradation, despite an earlier claim that Fe^{3+} was ineffective [15]. However, both ions exhibited less catalytic activity than Fe^{2+} at the same concentrations. Fe^{3+} was catalytic only at the lowest concentration studied, and although Cu^{+} was catalytic over the whole range, its effect increased with increasing concentration. Thus the two ions showed regular, but opposing, trends at these concentrations. It is also interesting to note that as with Fe^{2+} catalysis, nitrile and thiocyanate formation followed a similar pattern, whilst isothiocyanate was suppressed. Assuming that the data for formation of nitrile, thiocyanate and total products (Table 2) represent slopes of curves approaching maxima as observed with Fe^{2+} catalysis, then the maximum for Fe^{3+} would be at a lower concentration than for Fe^{2+}, whilst that for Cu^{+} would be at a higher concentration than for Fe^{2+}. It has been suggested above for Fe^{2+} catalysis that metal ions might function in glucosinolate degradation by complexing with ascorbate, and these data for ions of different oxidation states support this proposal. However, it has also been suggested that Fe^{3+} interferes with glucosinolate degradation by oxidizing ascorbate [19] and clearly this is also possible here.

However, whatever their mode of action, metal ions do considerably affect the course of glucosinolate degradation, generally promoting nitrile formation over isothiocyanate to a very great extent. Different metal ions may show different behaviour. More work of this type is obviously required, based on other natural systems and using other metal ions. The findings reported here have importance with regard to the nature and proportions of the products of glucosinolate degradation formed naturally in appropriate plant systems. They also have similar im-

Table 2. Amounts of benzylglucosinolate degradation products formed in *Lepidium sativum* seed (1.0 g) autolysates in the presence of varying concentrations of Fe^{3+} and Cu^{+}

Concentration of metal ion (M)	Benzyl cyanide		Benzyl thiocyanate		Benzyl isothiocyanate		Total products (mg)
	mg	%	mg	%	mg	%	
0 (Blank)	2.99	67.2	0.70	15.8	0.76	17.0	4.45
1×10^{-3} Fe^{3+}	5.14	79.3	0.86	13.3	0.48	7.4	6.48
8×10^{-3} Fe^{3+}	2.47	81.0	0.49	16.1	0.09	2.9	3.05
5×10^{-2} Fe^{3+}	1.25	82.5	0.22	14.5	0.05	3.0	1.52
1×10^{-3} Cu^{+}	3.85	83.3	0.46	9.9	0.31	6.8	4.62
8×10^{-3} Cu^{+}	4.45	88.1	0.46	9.0	0.15	2.9	5.06
5×10^{-2} Cu^{+}	4.63	83.8	0.47	8.5	0.42	7.7	5.52

portance with regard to plant systems which have been subjected to certain treatments involving metals, either deliberately or by accident. Thus it would be anticipated that a glucosinolate-containing food in contact with Fe^{2+}, Fe^{3+} or Cu^{+} (e.g. from machinery or container) would liberate unexpected and considerably increased levels of nitrile over otherwise normal generation of isothiocyanate. It has been conservatively estimated that the average per capita consumption of fresh Cruciferae vegetables (the major, but not exclusive, source of glucosinolates) is about 16 g/day, and that the consequent intake of glucosinolates is about 3 mg/person/day [29], but this latter estimate is dubious and almost certainly low in that it totally ignored glucosinolate catabolism to nitriles. Consideration should, therefore, be given to the toxicological and physiological effects of such nitriles which may be consumed regularly in certain foods at relatively high levels.

EXPERIMENTAL

Lepidium sativum ('curled cress') seeds were obtained from Suttons Seeds Ltd., Reading, U.K. and were authenticated by basic seed microscopy studies.

Seed autolysis. L. sativum seeds were ground to a fine powder in a coffee grinder. To 1.0 g seed powder was added dist. H_2O (50 ml) containing an accurately measured amount of $(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$ [or $Fe(NO_3)_3 \cdot 9H_2O$ or $(NH_2CSNH_2)_3CuCl$]. The mixture was shaken at room temp. for 15 min. The seed residue was removed by centrifugation and the aq. layer extracted with CH_2Cl_2 (2 × 30 ml) containing a measured amount (1.0 mg) of 3-phenylpropanal as int. standard. Following centrifugation the organic layer was separated, dried (Na_2SO_4) and carefully reduced in vol. to less than 5 ml using a rotary evaporator without the application of heat. Determinations were carried out in duplicate and agreed within ± 5%. The recovery of the procedure for benzyl cyanide, isothiocyanate and thiocyanate was assessed by the method of standard addition.

Analysis by GC. Extracts (1 μl) were examined by routine GC using a Pye-Unicam model 104 instrument with heated FID and a 1.5 m × 4 mm i.d. glass column packed with 3% Carbowax 20 M coated on 100–120 BSS mesh acid-washed Diatomite C. The carrier gas was N_2 (40 ml/min) and the column temp. was 160°C. An attenuation setting of 200 (i.e. 2×10^{-10} *A* full scale deflection) was necessary. RR_t*s* were measured from the onset of the solvent peak. Peak area measurements were accomplished manually. Absolute amounts of compounds produced were determined by standard injections of pure samples of benzyl cyanide, benzyl isothiocyanate and benzyl thiocyanate.

GC/MS. Glucosinolate degradation products in the samples were identified by GC/MS using a Kratos MS 25 instrument (in the EI mode) equipped with a Kratos DS 50S data processing system. The same GC conditions as above were used but with He as carrier gas. MS conditions were: ionization potential, 70 eV; ionization current, 300 μA; source temp., 230°; resolution, 600; scan speed, 3 sec/decade (repetitive throughout run).

Acknowledgements—We thank Mr. W. G. Gunn and Mr. A. E. Cakebread for carrying out the GC/MS, and Dr. J. G. Vaughan of the Botany Department, Queen Elizabeth College, for authenticating seeds of *L. sativum*.

REFERENCES

1. Hasapis, X. and MacLeod, A. J. (1982) *Phytochemistry* (in press).
2. Miller, H. E. (1965) M. A. thesis, Rice University, Houston, Texas, U.S.A.
3. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 2547.
4. Saarivirta, M. (1973) *Planta Med.* **24**, 112.
5. Cole, R. A. (1976) *Phytochemistry* **15**, 759.
6. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 227.
7. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 1369.
8. Kaoulla, N., MacLeod, A. J. and Gil, V. (1980) *Phytochemistry* **19**, 1053.
9. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 1657.
10. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 1365.
11. Benn, M. H. (1977) *Pure Appl. Chem.* **49**, 197.
12. Austin, F. L. and Gent, C. A. (1967) *Chem. Commun.* 71.
13. Youngs, C. G. and Perlin, A. S. (1967) *Can. J. Chem.* **45**, 1801.
14. Austin, F. L., Gent, C. A. and Wolff, I. A. (1968) *Can. J. Chem.* **46**, 1507.
15. Tookey, H. L. and Wolff, I. A. (1970) *Can. J. Biochem.* **48**, 1024.
16. Tookey, H. L. (1973) *Can. J. Biochem.* **51**, 1305.
17. Austin, F. L., Gent, C. A. and Wolff, I. A. (1968) *J. Agric. Food Chem.* **16**, 752.
18. Kirk, L. D., Mustakas, G. C., Griffin, E. L. and Booth, A. N. (1971) *J. Am. Oil Chem. Soc.* **48**, 845.
19. West, L. G., Badenhop, A. F. and McLaughlin, J. L. (1977) *J. Agric. Food Chem.* **25**, 1234.
20. MacLeod, A. J. and MacLeod, G. (1970) *J. Food Sci.* **35**, 739.
21. MacLeod, A. J. and MacLeod, G. (1970) *J. Food Sci.* **35**, 734.
22. MacLeod, A. J. and Pikk, H. E. (1979) *J. Food Sci.* **44**, 1183.
23. MacLeod, A. J., Panesar, S. S. and Gil, V. (1981) *Phytochemistry* **20**, 977.
24. Sato, T., Nagata, K., Shiro, M. and Koyama, H. (1966) *Chem. Commun.* 192.
25. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 2071.
26. Ohtsuru, M. and Hata, T. (1973) *Agric. Biol. Chem.* **37**, 1971.
27. Ohtsuru, M. and Hata, T. (1975) *Agric. Biol. Chem.* **39**, 1505.
28. Hasapis, X. and MacLeod, A. J. (1982) *Phytochemistry* **21**, 291.
29. Mullin. W. J. and Sahasrabudhe, M. R. (1978) *Nutr. Rep. Int.* **18**, 273.