*Phytochemistry*, 1975, Vol. 14, pp. 9–29. Pergamon Press. Printed in England.

## REVIEW

# ISOLATION AND CHARACTERIZATION OF NATURALLY OCCURRING CYANOGENIC COMPOUNDS

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(*Received* 15 *April* 1974)

**Key Word Index**—Cyanogenic glycosides; pseudocyanogenic glycosides; isolation; spectral properties; review.

**Abstract**—The literature dealing with the detection, isolation, purification and characterization of cyanogenic glycosides has been integrated with spectral and chemical data as well as other techniques from our laboratory to establish a method for the positive identification of glycosides of this type. The compounds are arranged into biosynthetically related groups (those derived from L-phenylalanine; L-tyrosine; L-leucine, L-valine; L-isoleucine; those with cyclopentene rings and pseudocyanogenic glycosides) and features of each of the above procedures are critically reviewed and spectral data for each group presented (IR, MS, UV and NMR). The NMR spectra of TMS ethers of cyanogenic glycosides have proven especially useful in chemical structure determination. This information is sufficient to permit identification of any of the 26 known glycosides as well as certain uncharacterized ones.

### INTRODUCTION

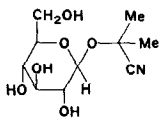
Many plants synthesize compounds which are capable of liberating hydrogen cyanide upon hydrolysis. This ability, known as cyanogenesis, has been recognized for centuries in such plants as apricots, peaches, almonds and other important food plants. Each year there are frequent livestock and occasional human victims of the many and widespread plants with cyanogenic ability. Most cases of cyanide poisoning are caused by the consumption of plants in the Rosaceae, Leguminosae, Euphorbiaceae (primarily cassava) or members of the genus *Sorghum* (Gramineae) [1]. Although many cases of poisoning are accidental (such as children drinking tea made of peach leaves or cattle eating young sorghum seedlings), large numbers of people are exposed daily to low concentrations of cyanogenic compounds in the foods they eat [2, 3]. Cassava flour in Nigeria may contain as much as 35 mg of HCN in the individual's approximate daily consumption (750 g), which represents almost one-half the lethal dose if consumed at one time. Although the body can detoxify relatively large amounts of HCN efficiently and rapidly, there is growing concern that certain neurological conditions arise from this chronic cyanide poisoning [2–5].

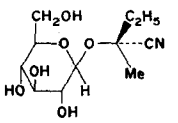
Two chemical types, cyanogenic glycosides and cyanolipids (e.g. **1** and **5**, Table 1) are responsible for this cyanophoric capability; both are derivatives of α-hydroxynitriles (cyanohydrins) and both liberate a carbonyl component and hydrogen cyanide when the sugar or the fatty acid moiety respectively is removed. The presence of hydrogen cyanide is readily detected by several simple and reasonably specific color tests [6–10]; these tests form the basis of most literature reports of cyanogenic plants. The isolation and characterization of cyanogens containing fatty acid moieties, which are much restricted in distribution, has been treated elsewhere [11, 12].

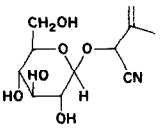
Cyanogenic glycosides are known to occur in at least 800 species of plants representing 70 to 80 families [10]. They are known from fungi, ferns, gymnosperms, both monocotyledonous and dicotyledonous angiosperms and several insects. In general, the highest concentrations are found in leaves, but compounds of interest may be concentrated in the roots, seeds or other plant tissues. Despite the fact that many plants are known to be cyanogenic, the structures of less than 30 such compounds have been reported, and the compounds responsible for activity have been studied in less than 50 species of plants. This is largely due

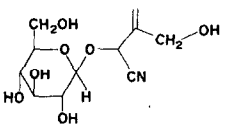
Table 1. Cyanogenic compounds from plants

A. *Derivatives of valine, isoleucine and leucine*

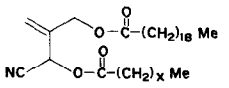

Linamarin (1)

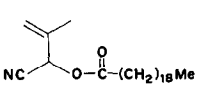

(*R*)-Lotaustralin (2)

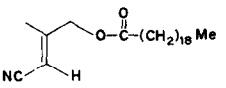

Acacipetalin (3)

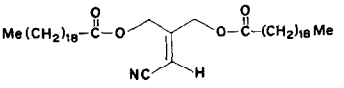

Cardiospermin (4)

Cyanolipids probably derived from leucine

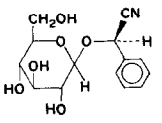

(5) x = 14, 16, 18, 20

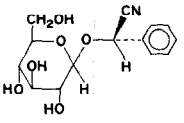

(6)

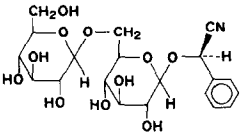

(7)*

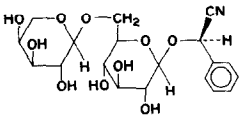

(8)*

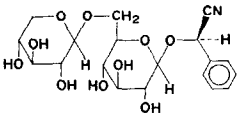
B. *Compounds derived from phenylalanine*


(*R*)-Prunasin (9)

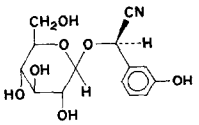

(*S*)-Sambunigrin (10)

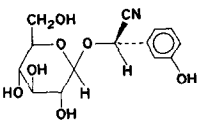

(*R*)-Amygdalin (11)


(*R*)-Vicianin (12)

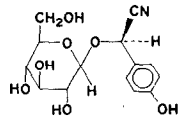

(*R*)-Lucumin (13)

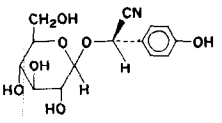
Probably derived from phenylalanine

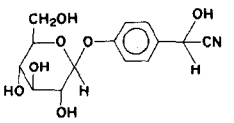

(*R*)-Holocalin † (14)

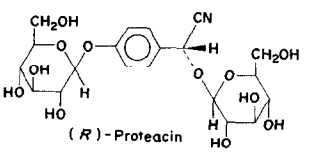

(*S*)-Zierin ‡ (15)

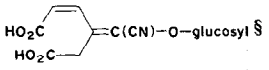
C. *Compounds derived from tyrosine*

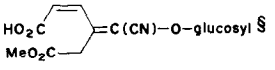

(*R*)-Taxiphyllin (16)


(*S*)-Dhurrin (17)

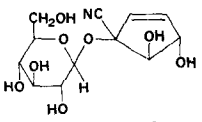

*p*-Glucosyloxymandelonitrile (18)

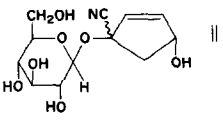

(*R*)-Proteacin

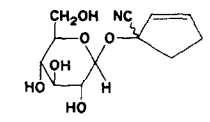

Triglochinin (20)


Methyl ester of triglochinin (21)

D. *Compounds with cyclopentene ring structure*


Gynocardin (22)

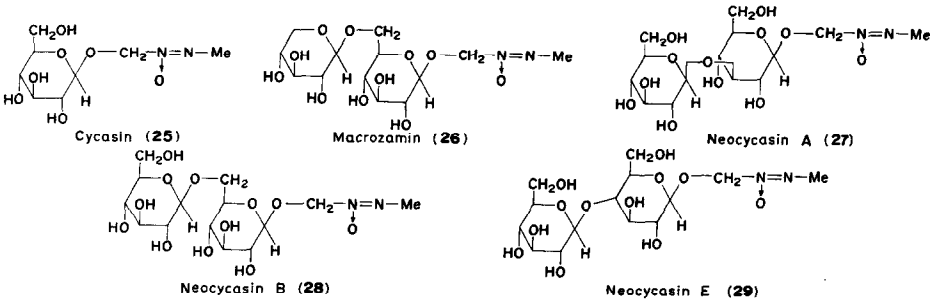

Barterin (tetraphyllin B) (23)


Deidaclin (tetraphyllin A) ¶ (24)

*continued*—

Table 1 (contd.)

*E. Pseudocyanogenic glycosides***




*Not cyanogenetic.
† See *Acta Chem. Scand.* **27**, 2661 (1973), and reference [61].
‡ Probably the (*S*) isomer of (*R*)-holocalin.
§ The position of the nitrile and the glucosyl group have not been established. The coupling constant (*J* 13 cps) for the two vinyl protons is consistent with either a *cis* or *trans* arrangement of the double bond.
The stereochemistry of the hydroxyl group has not been established but is probably the same as in gynocardin.
• It has not been established that tetraphyllin A and deidaclin are identical.
** The position of the oxygen in the azoxy group is not definitely established.

to difficulties in isolation and purification, as well as the inherent instability of the aglycones of some upon hydrolysis. Enzymes within the plant decompose these compounds on damage to the tissues in which they occur. Furthermore, sugars and other glycosides with which they co-occur often make purification difficult. Only one cyanogenic compound, amygdalin [11], is commercially available and reference standards of most others are difficult to obtain.

Because of the toxicological and chemical interest in cyanogenesis, we have attempted to develop more rapid and positive methods for the identification of this group of compounds. Through the kindness of numerous investigators and work in our own laboratory we have obtained samples of many cyanogenic compounds. We have tried to correlate as much data as possible derived from these samples, our own work and the literature to make this goal attainable. The NMR spectra of TMS (trimethylsilyl) ethers of the glycosides has proven especially valuable in this regard. Much information concerning the techniques of working with these compounds is scattered and recent reviews have dealt more with distribution, biosynthesis, toxicology and other aspects of this interesting group of compounds than the practicalities of isolation and identification.

## STRUCTURES AND PHYSICAL PROPERTIES

The structures of 29 currently known cyanogenic compounds are arranged by probable biosynthetic origin in Table 1, and several of their important physical properties are summarized in Table 2. In general, accurate melting points and optical rotations can only be obtained with pure samples. Glycosides are often hydrated and it is sometimes difficult to obtain crystalline materials with reproducible melting points. The corresponding acetates, which are soluble in less polar solvents, can usually be readily prepared and their melting points are more easily reproducible.

## DETECTION, ISOLATION AND PURIFICATION

In various isolation and purification procedures, it is necessary to ascertain those fractions containing cyanogenic glycosides. This may be accomplished by several techniques. One of the easiest is to hydrolyze a small portion of the fraction (from

Table 2. Physical properties of cyanogenic glycosides

| Compound | Formulae No. | m.p. (°C) | Optical rotation | Acetate derivatives | m.p. | References |
|---|---|---|---|---|---|---|
| Acacipetalin | **3** | 176–177 | $[\alpha]_D^{26}$ −36·6 (2, 0·25, $H_2O$) | Tetraacetate | 104 | [13] |
| Amygdalin | **11** | 214d | $[\alpha]_D^{20}$ −41·9 | Heptaacetate | 171–172 | [78, 81] |
| Barterin | **23** | 157–158 | $[\alpha]_D$ −78 (EtOH) | | | [14] |
| Cycasin | **25** | 145–146 | $[\alpha]_D$ −46·5 ($H_2O$) | | | [15] |
| Deidaclin | **24** | 127–128 | $[\alpha]_D^{27}$ −20·4 (c, 1, $H_2O$) | | | [16] |
| Dhurrin | **17** | 165 | $[\alpha]_D^{20}$ −62·7 (c, 0·485, EtOH) | Pentaacetate | 132–132·5 | [17] |
| *p*-Glucosyloxymandelonitrile | **18** | 160–164 | — | | | [18] |
| Gynocardin | **22** | 165–166 | $[\alpha]_D^{28}$ 72·9 (c, 0·96, $H_2O$) | Hexaacetate | 119–120 | [19] |
| Holocalin | **14** | 154–155 (impure, see ref. 61) | | Pentaacetate | 129–130 | [98] |
| Linamarin | **1** | 143–144 | $[\alpha]_D^{32}$ −28·5 (c, 3·86, $H_2O$) | Tetraacetate | 140–141 | [21, 22] |
| Lotaustralin | **2** | 123·5–124·5 | $[\alpha]_D^{25}$ −19·15 (c, 1, $H_2O$) | Tetraacetate | 116–116·5 | 183–184 |
| Lucumin | **13** | 183–184 | $[\alpha]_D$ −224 ($H_2O$) | Hexaacetate | 137–138 | [23] |
| Macrozamin | **26** | 199–200d | $[\alpha]_D^{16}$ −70 (c, 0·4, $H_2O$) | Hexaacetate | 150–151 | [24, 25] |
| Neocycasin A | **27** | 162–163d | $[\alpha]_D^{20}$ −35·1 | Heptaacetate | 142–143 | [26] |
| Proteacin | **19** | 102–104 | | Octaacetate | 181–183 remelted at 208–209 | [27] |
| Prunasin | **9** | 148–151 | $[\alpha]_D^{28}$ −30·1 (c, 0·418, $H_2O$) | Tetraacetate | 139–140 | [28, 29, 78] |
| Sambunigrin | **10** | 151–152 | $[\alpha]_D^{20}$ −76·1 | Tetraacetate | 125–126 | [78] |
| Taxiphyllin | **16** | 168–169d | $[\alpha]_D^{20}$ −66·7 (c, 0·372, EtOH) | Pentaacetate | 144–144·8 | [17] |
| Tetraphyllin A | **(24)** | 116–118 | $[\alpha]_D^{25}$ −14·0 (c, 1·0, $H_2O$) | Tetraacetate | 108–109 | [30] |
| Tetraphyllin B | **(23)** | 169–170 | $[\alpha]_D^{25}$ −35·6 (c, 1·0, $H_2O$) | Pentaacetate | 114–115 | [30] |
| Triglochinin | **20** | — | $[\alpha]_D^{16·5}$ +5·5 (c, 0·2, MeOH) | — | — | [31] |
| Vicianin | **12** | 175–176 | $[\alpha]_D^{19·1}$ −20·0 (c, 0·5, $H_2O$) | Hexaacetate | 170–171 | [32] |
| Zierin | **15** | 153–156 | $[\alpha]_D^{20}$ −29·5 | Pentaacetate | 117·5–118·5 | [98] |

a column, or from a paper or TLC) with enzyme, acid or base and perform one of the HCN color tests [6–10]. A sandwich method for the determination of cyanogenic compounds directly on paper chromatograms or cellulose TLC plates with picrate paper has been described [34]. Cyanogens may also be detected on paper of cellulose TLC plates by spraying with a 0·1 M *p*-nitrobenzaldehyde and 0·1 M *o*-dinitrobenzene solution in methyl cellosolve. After drying, the paper is sprayed with a 0·1% solution of β-glucosidase in phosphate buffer (pH 7·9). The paper is then placed on a polyethylene sheet on a sponge and a cover plate added. After about 30 min, the plate is removed and the paper sprayed with 2% NaOH in water–acetone (1:2). The paper is then dried at 80° for a short time; cyanogenic glycosides appear as violet-blue spots. The limit of detection

is about 5–10 $\mu$g [35]. This method is somewhat similar to that of Bennett and Tapper [84] which has been widely used.

It should be born in mind that some compounds, e.g. linamarin and lotaustralin, are hydrolyzed slowly by the $\beta$-glucosidase of almonds (emulsin) preparations. Additionally, the sensitivity of individual compounds to basic or acidic reagents varies widely and several different hydrolysis conditions may need to be examined to maximize the results of the HCN test. Once the appropriate spots are located on paper or thin layer chromatograms, they may be visualized with a number of reagents. Some which have proven generally useful are given in Table 3.

Cyanogenic glycosides are normally isolated from plant materials by grinding, with subsequent or concomitant extraction with solvents such as EtOH, MeOH, water or mixtures thereof. If fresh materials are used, it is desirable to freeze them with dry ice or liquid nitrogen to facilitate grinding. The mixture is heated to boiling (usually in 80% EtOH) for several minutes to further deactivate enzymes. The slurry is filtered and centrifuged to remove particulate material. The extraction method used by Reay [30, 40, 41] avoids some problems of enzymatic and possible thermal decomposition by carrying out the extraction at $-80°$, followed by filtration at room temp. Solvent is then removed under vacuum and the sample redissolved in water. Any precipitate which may occur at this stage should be removed by filtration and tested for HCN before being discarded. If the plant material was not initially extracted with a non-polar solvent to remove lipids, the aqueous solution should be extracted with light petroleum or $CHCl_3$.

From this point approaches differ greatly. A number of workers have used lead acetate treatment followed by hydrogen sulfide to remove undesirable acidic components. Others have used chromatography on polyvinylpyrrolidone to remove undesirable phenolic compounds [30, 31, 35, 40, 41]. Mixed bed ion exchange resins or a combination of acidic and basic ion exchange resins also remove many acidic, basic, and ionic impurities [42, 43] but caution should be exercised as some cyanogenic compounds, contain carboxyl groups. Eyjólfsson [31] used a basic ion exchange resin (Amberlite IR-45,$OH^-$) to isolate triglochinin which contains two carboxyl groups. Several workers have used kieselguhr, celite, diatomaceous earth, charcoal, carbon, cellulose or combinations of these for additional purification steps.

At this stage of purification, most cyanogenic compounds are reasonably stable; however they should be stored under refrigeration and under an inert atmosphere as a precautionary measure. These prepurified fractions are frequently contaminated with sugars and other glycosidic compounds and several additional purification methods are often required. Some compounds have been isolated and/or further purified by continuous liquid–

Table 3. Techniques for visualization of cyanogenic glycosides on paper and thin layer chromatograms*

| Reagent | Chromatogram | Compound |
|---|---|---|
| 0·1 M Alkaline $KMnO_4$ | Paper | Proteacin [18] |
| Naphthoresorcinol–$H_2SO_4$ reagent followed by heating at 105° | Silica gel | Lucumin [36]<br>Triglochinin [31] |
| $H_2SO_4$–water (1:1) and heating | Silica gel | Tetraphyllin A [30] |
| Resorcinol-hydrochloric acid in ethanol solution | Paper | Cycasin [26] |
| 2% $\alpha$-Naphthol in EtOH followed by conc $H_2SO_4$ and heating | Silica gel | Linamarin [22]<br>Lotaustralin |
| $AgNO_3$ in aqueous acetone, followed by 0·5 NaOH in EtOH–$H_2O$ | Silica gel or paper | Acacipetalin [37] |

* These methods detect sugars and glycosides other than cyanogenic derivatives; they do not measure the presence or absence of the nitrile function.

Table 4. Solvent systems used for paper chromatographic isolation and purification of cyanogenic glycosides

| Compound | $R_f \times 100$ in solvent system* | | | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| Acacipetalin | 73[a] | 67 | | | | | | | | [37, 45] |
| Amygdalin | 35[a] | 6 | 37 | 43[a] | | 72 | 60 | | 30[a] | [9, 29, 35] |
| Barterin | 60[b] | | | | 30[a] | | | | | [14] |
| Cardiospermin | 51[a] | | | | | | | | | [46] |
| Cycasin | | | | | 55[b] | | | | | [26] |
| Deidaclin | 85[a] | | 57, 82 | 57[a] | | | | | | [9, 47] |
| Dhurrin | 74[a] | 80 | | | | | | | 42[b] | [45] |
| *p*-Glucosyloxymandelonitrile | | | | 72[b] | 82[c] | | | | 65[c] | [18] |
| Gynocardin | 41 | | 41 | 42[a] | | 66 | 61 | | | [9] |
| Holocalin | | | | | 70[a] | | | | | [20] |
| Linamarin | 52[c] | 67 | 45, 54 | | | 77 | 71 | | | [9, 21, 40, 45] |
| Lotaustralin | 66[a] | 73 | 66 | 66[a] | | 83 | 71 | | 57[b] | [9, 21, 40, 45, 47] |
| Neocycasin A | | | | | 33[b] | | | | | [26] |
| Proteacin | | | | 35[b] | 85[c] | 55 | | | 38[c] | [18] |
| Prunasin | 91[a] | 83 | | 70[a] | | 85 | 78 | 78 | 70[a] | [9, 29, 35, 48] |
| Taxiphyllin | | | | | | | | 68 | | [48] |
| Tetraphyllin A + B | 71[d] | | | | | | | | | [80] |
| Triglochinin | | | | | | | | 66 | | [31, 48] |
| Triglochinin-methylester | | | | 70[b] | | | | | | [49] |

* Solvent systems 1: 2-Butanone–acetone–$H_2O$; (a) 15:5:3, (b) 30:11:6, (c) 15:5:2. 2: n-Butanol–pyridine–$H_2O$; 6:4:3. 3: EtOAc–acetone–$H_2O$; 4:5:1. 4: n-Butanol–EtOH–$H_2O$; (a), 7:2:2, (b) 40:11:19. 5: n-Butanol–HOAc–$H_2O$; (a) 4:1:5, (b) 4:1:1, (c) 12:3:5. 6: n-PrOH–$H_2O$; 7:3. 7: MeOH–$H_2O$; 9:1. 8: 2-Butanone–EtOAc–$HCO_2H$–$H_2O$; 5:3:2:1. 9: (a) wet BuOH on cellulose TLC, (b) n-BuOH–$H_2O$; 50:9, (c) 5% HOAc.

liquid extraction with ethyl acetate [28, 32, 36]. Others have been purified by paper chromatography; some of the solvent systems used and $R_f$ values of glycosides are given in Table 4. Triglochinin [31], cycasin [43], prunasin [35], and proteacin [27] have all been purified by chromatography on cellulose columns. Silica gel (and to a lesser extent kieselgel and Florisil) have been used for final purification of cyanogenic compounds; the pertinent data for several of these separations are presented in Table 5.

It is possible to separate the TMS ethers of most cyanogenic compounds from co-occurring impurities by GC, although generally poor results are obtained with crude samples. Samples which have been purified by one of the previously described methods normally give excellent results. Several separations which have been accomplished are de-

Table 5. Purification of cyanogenic compounds on silica gel, kieselgel, and florisil*

| Compound | Support | Form | Solvent system |
|---|---|---|---|
| Acacipetalin | Silica gel | Column | PrOH–$H_2O$ mixtures [37] |
| Cardiospermin | Silica gel | Column | PrOH–$H_2O$ mixtures [46] |
| Dhurrin | Silica gel | TLC | 2-butanone–EtOAc–$HCO_2H$ water (5:3:2:1) [17] |
| Gynocardin | Silica gel | Column | Acetone [19] |
| Holocalin | Kieselgel | Column | EtOAc–MeOH mixture [20] |
| Linamarin | Silica gel | Column | $CHCl_3$–MeOH (5:1) [22] |
| | | TLC | $CHCl_3$–MeOH* (5:1) [21] |
| Lotaustralin | Silica gel | Column | $CHCl_3$–MeOH (5:1) [21] |
| | | TLC | $CHCl_3$–MeOH* (5:1) [21] |
| Lucumin | Silica gel | Column | EtOAc–MeOH–water (7·9–1·1–1) [36] |
| Prunasin | Silica gel | Column | MeOH–EtOAc mixtures [32] |
| Tetraphyllin A | Silicic acid | Column | $CHCl_3$–MeOH mixtures [30] |
| | Florisil | Column | $CHCl_3$–MeOH (9:1) [30] |
| | Silica gel | TLC | $CHCl_3$–MeOH (4:3) [30] |
| Tetraphyllin B | Silicic acid | Column | $CHCl_3$–MeOH mixtures [30] |
| Vicianin | Silica gel | Column | MeOH–EtOAc mixtures [32] |

* $R_f$ lotaustralin/$R_f$ linamarin = 1·2 in $CHCl_3$–MeOH (5:1).

Table 6. Gas chromatographic separations of the TMS ethers of cyanogenic glycosides

| Column liquid phase | Temperature (°C) | Support | Compounds (as TMS ethers) and references |
|---|---|---|---|
| 3% OV-1 | 238 | Gaschrom Z | Cycasin [15] |
| | 200 | Chromasorb W | Cycasin [15] |
| | 190–280, 1°/min | Chromasorb AW DMCS | Prunasin and sambunigrin [85] |
| | 190–280, 1°/min | Chromasorb AW DMCS | Amygdalin and neoamygdalin [85] |
| | 150–210, 5°/min | Gaschrom Q | Acacipetalin [37] |
| | 150–210, 5°/min | Gaschrom Q | Cardiospermin [46] |
| | 150–250, 5°/min | Gaschrom Q | Lotaustralin linamarin [50] |
| 3% ECNSS-M | 155° | Gaschrom Q | Prunasin and sambunigrin [35] |
| 10 or 20% SE-30 | 210–230° | Silanized Chromasorb W | Linamarin and lotaustralin [21] |
| 3% OV-17 | 195–255° | Chromasorb AW DMCS | Prunasin and sambunigrin [85] |
| | 195–255° | Chromasorb AW DMCS | Amygdalin and neoamygdalin [85] |
| 3% SE-30 | 215° (22 min) followed by 6°/min | Chromasorb AW DMCS | Deidaclin [47] |

scribed in Table 6. Corresponding diastereomers are not separated on SE-30, but may be resolved on OV-1, OV-17, and other liquid phases [44]. Nahrstedt [21] observed that in the series prunasin-sambunigrin, vicianin-isovicianin, lucumin-isolucumin, and amygdalin-neoamygdalin, the (*S*)-form is eluted first from the column. Lotaustralin has been isolated by preparative GC of the TMS ether on SE-30 silicone gum liquid phase.

## SPECTRAL PROPERTIES

After the materials of interest have been isolated and purified, one may make spectral determinations to identify the cyanogenic compounds. These measurements involve relatively small amounts of materials which can often be reclaimed for further testing.

Although determination of IR spectra would seem to be a potential method for monitoring fractionations, nitrile absorptions are quenched in cyanohydrins and their derivatives by the presence of an oxygen on the nitrile bearing carbon [38, 39].

For determination of an NMR spectrum on a 60 mc machine about 20–30 mg is required; for a 100 mc instrument good spectra can often be obtained with as little as 1–5 mg. The spectrum may be determined by several procedures. They may be run directly in $D_2O$, as most of the compounds involved are water-soluble. However, spectra determined in heavy water have somewhat broadened peaks as compared to those measured in non-polar solvents, and the presence of an HDO peak (near 4·6 $\delta$) often overlaps peaks of interest in cyanogenic compounds.

The NMR spectra of TMS ethers of cyanogenic glycosides in non-polar solvents such as $CCl_4$ and

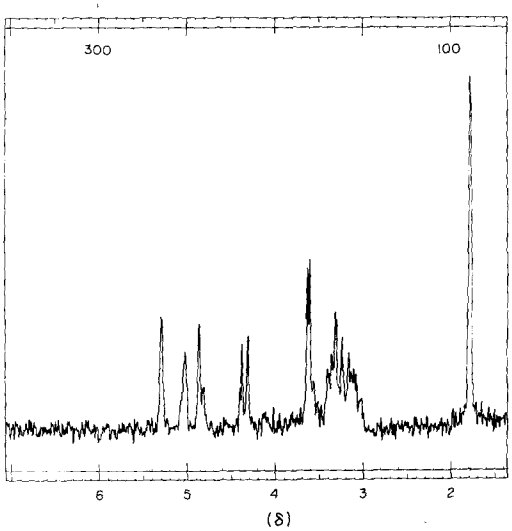



Fig. 1. The NMR spectrum of the TMS ether of acacipetalin in $CCl_4$.

$CDCl_3$ have proven especially useful in our laboratory for the characterization and identification of these compounds (Fig. 1). To prepare these derivatives we have slightly modified the procedure of Mabry *et al.* [51]. This procedure has many advantages: it is fast, inexpensive, and relatively free of side reactions.

Amygdalin (10 mg) was dissolved in pyridine (stored over KOH, 0·5 ml) in a small vial. It is important to have the compound in soln at this point. Slight warming may be necessary, but extensive heating with pyridine may cause racemization.* Hexamethyldisilazane (HMDS) and trichloromethylsilane (TMCS) (0·5 ml, Pierce Chemical Co., Rockford, Illinois) are added and the vial warmed gently for about 15 min. The mixture is then conc under a stream of $N_2$ and the remaining paste dried under vacuum (oil pump). The dried material is then scraped from the sides with a spatula, and thoroughly dispersed in $CCl_4$ (1–2 ml). This mixture is filtered through a small pipette plugged with glass wool (not cotton) and the filtrate conc under $N_2$. The residue is redissolved in $CCl_4$ (0·5 ml) which contains tetramethylsilane (1%) and transferred to a dry NMR tube. HMDS and TMCS (1 drop each) are added to insure anhydrous conditions and the sample is ready for NMR analysis.

The use of unextracted cotton for filtration should be avoided, and the lower half of each NMR tube should be rinsed with chloroform before determination of the spectrum to avoid contamination with lipids. These lipid impurities often produce peaks at 1·25 and 0·9 $\delta$ in spectra of TMS ethers. To recover the cyanogenic compounds, add MeOH (two or three drops) and allow the solvent to evaporate most of the compounds we have tested can be recovered, apparently unchanged, although there is possible danger of racemization.

Acetates are generally more difficult to prepare than TMS ethers. These, however, are crystalline, water-stable solids that can be preserved more easily and, in contrast to the glycosides, can be dissolved in non-aqueous solvents, hence are easier to separate from residual amounts of water and give sharper and more reproducible melting points. The two procedures in Shriner *et al.* [53] work well for most of the compounds we have tested. One can often obtain a good NMR spectrum on non-crystalline material. The NMR spectra of acetates are usually determined in $CDCl_3$. Most syntheses of cyanogenic glycosides involve preparation of the tetraacetates of cyanogenic glycosides (see Table 15). For comparison of the naturally occurring material and the synthetic product, it may be easier to convert the natural product to the corresponding acetate than to remove the acetates from the synthetic product.

Several biochemical applications of mass spectrometry have recently been reviewed [54]. A number of workers have used mass spectrometry of cyanogenic compounds and their derivatives, e.g. acetates and TMS ethers, to establish MWs and to identify aglycone portions of the molecules by interpretation of their fragmentation patterns [16, 19, 21, 30, 31, 37, 47, 90].

### PSEUDOCYANOGENIC GLYCOSIDES

Several derivatives of methylazoxymethanol (so-called pseudocyanogenic glycosides) are known to occur in the seeds of members of the family Cycadaceae. These compounds, which are of considerable interest since cycad seeds are used as a source of edible starch in some parts of the world [43], cause livestock fatalities in Australia [55], are thought to be related to the high incidence of amyotrophic lateral sclerosis in Guam [56], and produce hepatomas [43].

When treated with dilute base these compounds liberate HCN, whereas treatment with acid or emulsin liberates $N_2$, formaldehyde and MeOH. They have a UV absorption at 217 nm which corresponds to that of the aglycone [43]. The presence of a strong IR absorption at about 1530 $cm^{-1}$ is associated with the azoxy group of the aglycone [24, 25].

Peaks corresponding to the methyl and methylene peaks of the aglycone portion of cycasin and neocycasin A are sharp singlets with integrals in a 3:2 ratio, (Table 7). Other protons correspond to those normally found in glycosides. One anomeric proton of laminaribiose is found at almost the same chemical shift value as that of glucose whereas the other falls partially under the methylene peak of the aglycone, and although it exhibits this relatively large chemical shift, it still falls in the range of $\beta$-glycoside linkages. The coupling constant also precludes the presence of an $\alpha$-linkage [57].

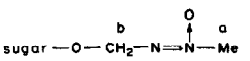
### CYANOGENIC COMPOUNDS DERIVED FROM TYROSINE

Several cyanogenic compounds have structures suggesting they are derived from tyrosine. This has been established for dhurrin, *p*-glucosyloxymandelonitrile, taxiphyllin, proteacin, triglochinin [58],

* This is normally a minor problem under the conditions used; see ref. 44 for example.

Table 7. NMR spectral data for compounds related to cycasin

| | Signal from protons* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1a | 2a | 1b | 2b | 1c | 2c | 2d | 1e | 2e | 1f | 2f |
| Chemical shift ($\delta$) | 3·92 | 3·96 | 4·87 | 4·92 | 4·34 | 4·36 | 4·9 | 3·66 | 3·70 | 2·8–3·6 | 2·8–3·7 |
| Number of protons | 3 | 3 | 2 | 2 | 1 | 1 | 1 | 2 | +2f 12 | 4 | +2e 12 |
| Multiplicity | *s* | *s* | *s* | *s* | *d* | *d* | *d* | *d* | *d* | complex multiplet | |
| Coupling constant (Hz) | — | — | — | — | 6·8 | 7·0 | — | 2·8 | — | — | — |

* Protons shown in formula  : 1. cycasin; 2. neocycasin A with c. anomeric proton of sugar; d. second anomeric proton of disaccharide; e. $-CH_2OTMS$ of sugar; and f. other sugar protons.

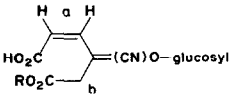
and the methyl ester of triglochinin [4, 59]. The distribution of these compounds is widespread in the plant kingdom: dhurrin is found in the Gramineae, taxiphyllin in the Taxaceae, Proteaceae and Euphorbiaceae; *p*-glucosyloxymandelonitrile in the Berberidaceae and Ranunculaceae; proteacin in the Proteaceae and Ranunulaceae; triglochinin in the Lilaeaceae and Juncaginaceae; and the methyl ester of triglochinin in the Ranunculaceae.

All compounds in this group have UV absorptions characteristic of *para* substituted phenols. The band at 220–230 nm ($\pi \rightarrow \pi^*$) generally has an E.C. of $6–10 \times 10^3$ whereas that at 270–280 nm ($n \rightarrow \pi^*$) is $0·2–2·5 \times 10^3$ [39]. Triglochinin and its methyl ester, although lacking an aromatic ring have an extended conjugated system and have absorptions at 275 and 280 nm respectively [18, 31]. Literature reports [17, 18] indicate that dhurrin has an absorption at 228 nm, *p*-glucosyloxymandelonitrile at 270, proteacin at 226 and 270, and taxiphyllin at 228 and 272 nm. Upon treatment with base, the absorption maxima undergo a bathochromic shift of about 25 nm in dhurrin and taxiphyllin; however these compounds undergo decomposition in base with time. This shift is not observed in proteacin and *p*-glucosyloxymandelonitrile, although the latter of these two compounds is sensitive to dilute acid and spontaneously liberates HCN.

The IR spectra of dhurrin and taxiphyllin have peaks at 3200–3480 (*s*, hydroxyl), 2860–2960 (*m*, C–H stretching), 1600 and 1515 (*m*, phenyl nucleus), 1622 (*s*, water of crystallization) 1020–1090 (*s*, ethereal stretching) and 840 $cm^{-1}$ (1,4-disubstituted benzene ring). Dhurrin has, in addition, absorptions at 920, 900 and 805 $cm^{-1}$ and taxiphyllin at

Table 8. NMR data for the acetate of triglochinin and that of its methyl ester measured in $CDCl_3$ [31, 56]

| | Signal from protons* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1a | 2a | 1b | 2b | 1c | 2c | 1d | 2d | 2e | 2f | 2g |
| Chemical shift ($\delta$) | 6·93 6·69 | 7·03 6·42 | 3·63 | 3·8 | 4·8 | under 2d | 4·1 – 2·9 | 5·14 | 3·8 | 5·4 | 4·2 |
| Integral value | 1 + 1 | 1 + 1 | ~2 | +2e 5 | 1 | — | 8 | 4 | +2b 5 | 2 | — |
| Multiplicity | two doublets | | *s* | *s* | *m* | — | complex mult. | broad *d* | *s* | broad signal | broad *d* |
| Coupling constant (Hz) | 12·5 | 13 | — | — | — | — | — | — | — | — | — |

* Protons shown in formula  : R = H, triglochinin; R = Me, methyl ester of triglochinin; c. anomeric proton of sugar; d. other sugar protons; e. methyl of the ester group; f. carboxyl proton; g. $-CH_2-OAc$ protons.

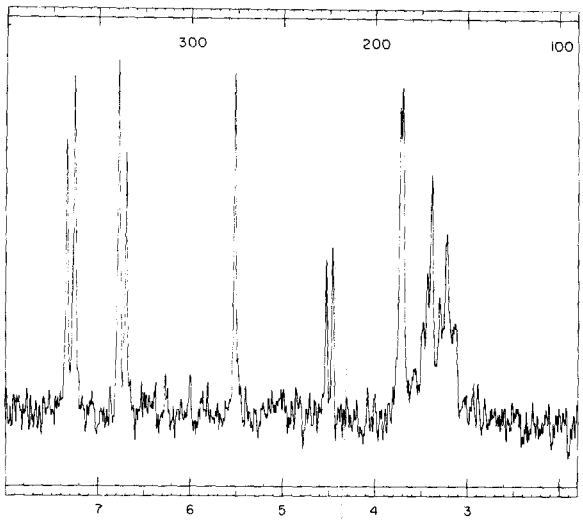



Fig. 2. The NMR spectrum of the TMS ether of dhurrin.

975, 950 and 900 $cm^{-1}$ (all medium, also see reference 22). The IR spectrum of triglochinin [31] has peaks at 1715 (acid carbonyl), 1625 (conjugated double bond), 1410 (active methylene) and 2220 $cm^{-1}$ (moderately strong, $\alpha$, $\beta$-unsaturated nitrile). Other peaks occur at 1200 (*s*), 1075 (*s*), 987 (*m*), 895 (*w*), 825 (*w*), and 632 (*w*) $cm^{-1}$. The corresponding methyl ester from *Thalictrum aquilegifolium* (Ranunculaceae) has peaks at 2220 (*m*), 1750 (*s*), 1230 (*m*), 1760 (*m*), 1650 (*m*), 1590 (*m*), 1030 (*m*), 800 (*m*), 720 (*s*) and 680 (*s*) $cm^{-1}$ suggesting a compound similar to triglochinin [49]. MS determinations have also been useful in determining structure. Thus, triglochinin was treated with anhydrous HCl–MeOH (prepared by adding acetyl chloride to MeOH) to yield two products; one of them was the dimethyl ester corresponding to triglochinin. The MS of this has a weak parent ($M^+$) ion at 387, and a prominent signal at *m/e* 162 ($M-C_6H_{10}O_5$)[31]. The methyl ester of triglochinin, after acetylation, gave peaks at *m/e* 210 ($M^+$ tetracetyl glucose), 168 and 151. When the glucoside acetate was methylated under conditions in which only the carboxylic acid could be methylated and the MS re-examined, the peak at 210 disappears and one is seen at *m/e* 224 [49].

A central argument in the structure determination of triglochinin was obtained using NMR. The coupling constant of the vinyl protons (5·93 and 6·69 $\delta$) [31, 56] was the same as is observed in the methyl ester isolated by Sharples *et al.* [49] (Table 8). This information together with the UV and IR spectral data strongly suggests that their compound is the methyl ester of triglochinin.

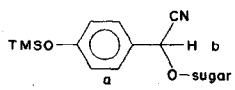
The NMR spectra of the pentaacetates of dhurrin and taxiphyllin have previously been examined [17]. In dhurrin the anomeric and methine protons were observed to be shifted downfield with respect to those of taxiphyllin in accord with theoretical predictions helping to establish the absolute configurations of the two compounds. In the spectra of the two TMS ethers (Fig. 2, Table 9), similar shifts were observed; however, the remaining sugar peaks were separated from the peaks of interest.

## CYANOGENIC COMPOUNDS DERIVED FROM PHENYLALANINE

Another major group of cyanogenic compounds is derived from phenylalanine. This route of bio-

Table 9. NMR data for the TMS ethers of dhurrin and taxiphyllin

| | Signal from protons* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1a | 2a | 1b | 2b | 1c | 2c | 1d | 2d | 1e | 2e |
| Chemical shift ($\delta$) | 7·31–6·73 | 7·37–6·77 | 5·52 | 5·36 | 4·50 | 3·96 | 3·72 | 3·69 | 3·1–3·6 | 2·9–3·7 |
| Number of protons | 4 | 4 | 1 | 1 | 1 | 1 | 2 | 2 | 4 | 4 |
| Multiplicity | $A'_2B'_2$ multiplet | | *s* | *s* | *d* | *d* | *d* | *d* | complex multiplet | |
| Coupling constant (Hz) | 8·0 | 8·0 | — | — | 7·0 | 7·0 | 3·0 | 3·0 | — | — |

* Protons shown in formula 

: 1. dhurrin; 2. taxiphyllin: c. anomeric proton of sugar; d. $-CH_2-OTMS$ of sugar; e. other sugar protons.

Table 10. The taxonomic distribution of cyanogenic glycosides derived from phenylalanine

| Compound | Family | Compound | Family |
|---|---|---|---|
| Prunasin | Rosaceae | Sambunigrin | Caprifoliaceae |
| | Saxifragaceae | | Leguminosae |
| | Myrtaceae | | Olacaceae |
| | Polypodiaceae | Vicianin | Leguminosae |
| | Myoporaceae | | Polypodiaceae |
| | Scrophulariaceae | Lucumin | Sapotaceae |
| | Caprifoliaceae | | |
| Amygdalin | Rosaceae | Zierin | Rutaceae |
| | | | Caprifoliaceae |
| Holocalin | Leguminosae | | |
| | Caprifoliaceae | | |

synthesis has been established for prunasin, amygdalin, and vicianin, and is likely for lucumin and sambunigrin [4]. It seems more probable that zierin and holocalin arise by oxidation of an intermediate derived from phenylalanine than descending from *m*-tyrosine. The distribution of these compounds is indicated in Table 10.

All compounds in this group have UV absorptions in the same range as substituted benzenes, except for holocalin in which the absorption maxima are at 222 and 280 nm ($\epsilon$ of 3300 and 2180 respectively) and resemble those of *m*-disubstituted phenols [20].

The IR spectra of cyanogenic compounds derived from phenylalanine show absorptions characteristic of mono-unsubstituted rings (745, 690–700 $cm^{-1}$), with the exception of holocalin with 785 and 705 $cm^{-1}$w (characteristic of *meta* substitution) [60]. All showed bands at 3200–3600 (hydroxyl), 2920–2980 (*m*, C–H stretching), and 1040–1095 $cm^{-1}$ (ethereal stretching bands). Only holocalin had a strong band at 1620 (water of crystallization). The nitrile absorption bands in prunasin (2270 $cm^{-1}$), and lucumin (2250 $cm^{-1}$), are barely detectable.

The NMR spectra are similar in many respects to those of compounds derived from tyrosine. The aromatic ring protons (in all except holocalin and zierin) are a broad multiplet at 7·45 (Figs. 3, 4, Table 11a and b). It has previously been demonstrated that lucumin, amygdalin and vicianin yield prunasin on partial hydrolysis [37], and thus have

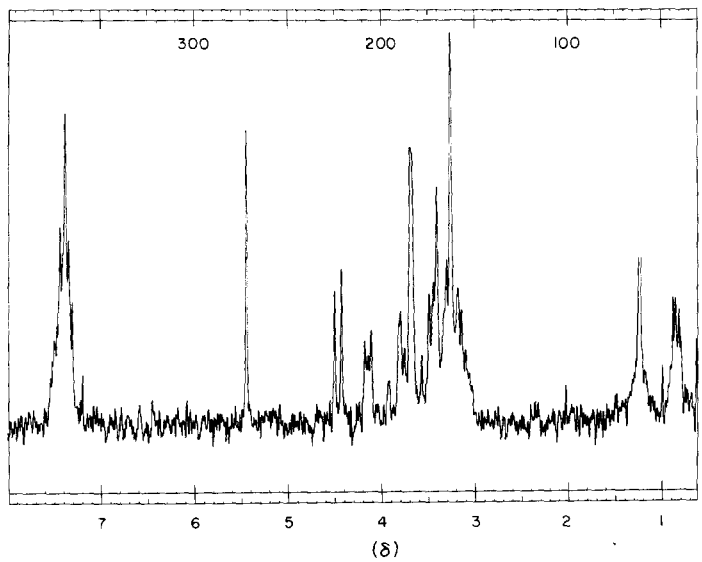



Fig. 3. The NMR spectrum of the TMS ether of amygdalin.

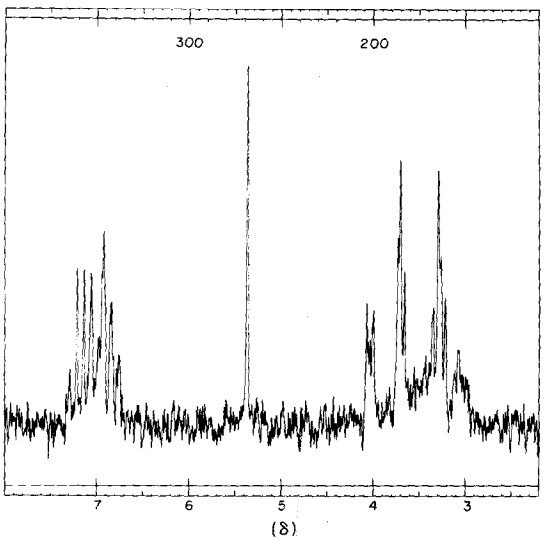



Fig. 4. The NMR spectrum of the TMS ether of holocalin.

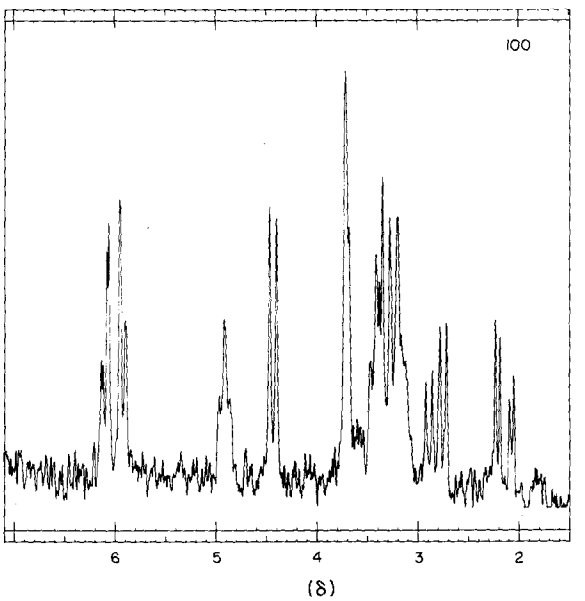



Fig. 5. The NMR spectrum of the TMS ether of tetraphyllin B.

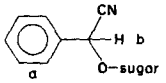
the same stereochemistry at the carbon of the cyanohydrin group (*R*). The NMR absorptions of the anomeric protons of prunasin (4·04), lucumin (4·12 and 4·32) and amygdalin (4·16 and 4·44) are all reasonably similar. The methine protons of prunasin (5·43), lucumin (5·44) and amygdalin (5·46) are consistently positioned. These peaks for the three glycosides all differ in the same direction from taxiphyllin which has an (*R*) configuration.

Gmelin has argued that since the rotation of holocalin is $[\alpha]_D - 59$ (compare: $[\alpha]_D - 76$ in sambunigrin and $[\alpha]_D - 65$ in dhurrin), this compound must have the (*S*)-configuration. He further states

Table 11a. NMR data of the TMS ethers of compounds derived from phenylalanine

| | Signal for protons* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1a | 2a | 3a | 4a | 1b | 2b | 3b | 4b | 1c | 2c | 3c | 4c |
| Chemical shift (δ) | 7·42 | 7·39 | 7·35 | 6·7–7·4 | 5·43 | 5·46 | 5·44 | 5·37 | 3·99 | 4·44 | 4·32 | 4·04 |
| Number of protons | 5 | 5 | 5 | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Multiplicity | complex multiplet | | | | *s* | *s* | *s* | *s* | *d* | — | *d* | *d* |
| Coupling constant (Hz) | — | — | — | — | — | — | — | — | 7·0 | 7·0 | 7·0 | 7·0 |

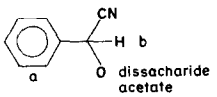
| | Signal for protons* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1d | 2d | 3d | 4d | 1e | 2e | 3e | 4e | 2f | 3f |
| Chemical shift (δ) | 3·68 | 3·69 | 3·72 | 3·72 | 2·9–3·9 | 3·0–3·9 | 3·0–3·8 | 2·9–3·6 | 4·16 | 4·12 |
| Number of protons | 2 | 2 | | 2 | 4 | +2d 12 | 11 | 4 | 4 | 1 |
| Multiplicity | *m* | *s* | *m* | *m* | complex pattern | | | | d | d |
| Coupling constant (Hz) | — | — | — | — | — | — | — | — | 6·5 | 6·0 |

* Protons shown in formula 

: 1. prunasin (glucose); 2. amygdalin (gentiobiose); 3. lucumin (prime-verose); 4. holocalin (glucose); c. anomeric proton of sugar; d. $-CH_2OTMS$ of sugar; e. other sugar protons; f. second anomeric proton of disaccharides.

Table 11b. NMR data for amygdalin heptaacetate and vicianin hexaacetate in $CDCl_3$ [32]

| | Signal for protons* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1a | 2a | 1b | 2b | 1c | 2c | 1d | 2d |
| Chemical shift ($\delta$) | 7·43 | 7·48 | 5·53 | 5·60 | 3·5–5·4 | 3·5–5·4 | 1·9–2·15 | 1·9–2·15 |
| Number of protons | 5 | 5 | 1 | 1 | 14 | 13 | 18 | 21 |
| Multiplicity | *s* | *s* | *s* | *s* | *m* | *m* | all *s* (4) | all *s* (5) |
| Coupling constant (Hz) | — | — | — | — | — | — | — | — |

* Protons shown in formula 

: 1. amygdalin (gentiobiose); 2. vicianin (vicianose); c. other sugar protons; d. acetate protons.

that it is a disastereomer of zierin which has the rotation $[\alpha]_D$ −29 (compare: prunasin $[\alpha]_D$ −30, taxiphyllin $[\alpha]_D$ −67). The proton of holocalin (3·99) is more similar in chemical shift to that of taxiphyllin (3·96) than to that of dhurrin (4·50 $\delta$); other effects such as *m* vs. *p* substitution may play a role but these appear to be small, when one compares the para and unsubstituted series. The methine proton of holocalin (5·37) is also closer to that of taxiphyllin (5·36) than that of dhurrin (5·52 $\delta$). Recent work by Nahrstedt[61] with GC separations of diastereomers indicates that holocalin has an (*R*)-configuration, in agreement with our NMR data [98]. The *m*-substituted ring pattern of holocalin is similar to those of other similarly substituted compounds [62].

Although the signals of the second anomeric protons of lucumin and amygdalin are shifted downfield with respect to the anomeric protons adjacent to the aglycone; the coupling constants (~7 Hz) and chemical shifts (4·32 and 4·44 $\delta$ respectively) indicated they represent $\beta$-linkages, and the values are similar to those for the glycosidic linkages of disaccharides (e.g. gentiobiose in $D_2O$ 4·50 $\delta$, *J* 7·7 Hz) [57]. The NMR spectra of amygdalin and vicianin are almost identical [32].

### CYANOGENIC COMPOUNDS DERIVED FROM VALINE, LEUCINE AND ISOLEUCINE

Several aliphatic cyanogenic compounds are derived from valine, leucine and isoleucine. In addition, the structures of all known cyanolipids [12] suggest they are derived from leucine. Linamarin and lotaustralin, frequently, if not always, co-occur and are found in the Compositae, Leguminosae, Euphorbiaceae, Linaceae and Papaveraceae. Acacipetalin is found only in two South African species of *Acacia* (Leguminosae) and cardiospermin only in the Sapindaceae.

Linamarin has IR absorptions at 3200–3600 (*s*, hydroxyl), 2940–3000 (*m*, C–H stretching), 1010–1190 (*s*, ether stretching) and 895 (*w*), 870 (*w*) and 705 (*w*). The use of the latter three bands to establish the nature ($\alpha$ or $\beta$) of the glycoside linkage of these compounds has been investigated by Clapp *et al.* [22]. The major absorptions of cardiospermin are similar, with the exception of a strong band at 1390 (*s*, vinyl) [46].

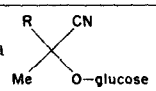
Bissett and coworkers identified lotaustralin (isolated as the TMS ether by gas chromatography) by comparison of its MS to an authentic sample of natural lotaustralin. The spectrum had peaks at *m/e* 535 (M-15) and 200 [$(Me)_3\overset{+}{S}iO$–OH =O–C(CN) (Me) $C_2H_5$]. These assignments were confirmed by high resolution mass spectrometry [21]. The MS of the TMS ether of linamarin showed analogous peaks at *m/e* 520 and 186. Both spectra had major fragments at *m/e* 73, 103, 147, 204 and 217 which are characteristic of the TMS ethers of glycosides [21, 63]. The MS of acacipetalin showed a parent ion at *m/e* 259 which was confirmed by accurate mass measurement. Other major fragments corresponded to *m/e* 163 (M-aglycone), 145 and 127 [37].

The NMR spectra of this group have been especially useful in determination of the aglycones, as the aglycones tend to be somewhat unstable under many reaction conditions (Table 12). The NMR spectra of linamarin and lotaustralin are reasonably straightforward. Linamarin and lotaustralin have a methyl singlet (1·52 and 1·58 $\delta$ respectively). In addition, lotaustralin has the characteristic of

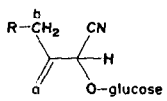
Table 12. NMR spectral data from the TMS ethers of compounds derived from valine, leucine and isoleucine

| | Signals for protons* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1a | 2a | 1-Me | 2-$CH_2$Me | 1c | 2c | 1d | 2d | 1e | 2e |
| Chemical shift ($\delta$) | 1·58 | 1·52 | 1·58 | 1·78 | 1·03 | 4·45 | 4·45 | 3·67 | 3·67 | 2·9–3·4 | 2·9–3·4 |
| Integral value | 3 | 3 | 3 | 2 | 3 | 1 | 1 | 2 | 2 | 4 | 4 |
| Multiplicity | *s* | *s* | *s* | *q* | *t* | *d* | *d* | *m* | *m* | complex pattern | |
| Coupling constant (Hz) | — | — | — | — | 7·0 | 7·0 | 7·0 | — | — | — | — |

| | Signals for protons† | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1a | 2a | 1b | 2b | 1c | 2c | 1d | 2d | 1e | 2e | 1f | 2f |
| Chemical shift ($\delta$) | 5·04<br>4·86 | 5·39<br>5·11 | 1·78 | 4·13 | 4·35 | 4·44 | 3·62 | 3·70 | 3·0–<br>3·5 | 3·1–<br>3·8 | 5·29 | 5·51 |
| Integral value | 2 | 2 | 3 | 2 | 1 | 1 | 2 | 2 | 4 | +2d<br>6 | 1 | 1 |
| Multiplicity | *s* | *s* | *s* | AB<br>*m* | *d* | *d* | *d* | *m* | complex pattern | | *s* | *s* |
| Coupling constant (Hz) | — | — | — | — | 7·0 | 7·0 | 3 | — | — | — | — | — |

* Protons shown in formula 

: 1. R = Me, linamarin; 2. R = $CH_2Me$, lotaustralin; c. anomeric proton of sugar; d. $-CH_2OTMS$ of sugar; e. other sugar protons.

† Protons shown in formula 

: 1. R = H, acacipetalin; 2, R = OTMS, cardiospermin; c. anomeric proton of sugar; d. $-CH_2OTMS$ of sugar; e. other sugar protons; f. methine proton of cyanohydrin.

an ethyl group pattern (triplet at 1·03 and a quadruplet at 1·78 $\delta$). The doublet corresponding to the anomeric proton of each is centered at 4·45 $\delta$ ($J$ 7·2 Hz) and 5·38 $\delta$ ($J$ 3·2 Hz) respectively, clearly indicating linamarin is a $\beta$-glucoside [22]. The (*R*) configuration of lotaustralin has been established by conversion to (−)2-hydroxy-2-methylbutyric acid; *epi*-lotaustralin gives the (+) acid. It has previously been demonstrated that the (−) acid has the (*R*) configuration [21].

The NMR absorptions of acacipetalin closely resemble those of the cyanolipid from *Ungnadia speciosa* (Sapindaceae) [64]. The spectrum has a methine proton (allylic in this case) at 5·29 $\delta$ (cyanolipid 5·79), two vinyl protons (5·04 and 4·86 $\delta$, cyanolipid 5·37 and 5·17 $\delta$) and a methyl group at 1·79 (cyanolipid 1·86). Irradiation of any one of these four signals results in decoupling of the other three, a characteristic of allylic systems [50]. The anomeric proton occurs at 4·35 $\delta$ ($J$ 7·0 Hz), indicative of a $\beta$-glycoside.

Cardiospermin is similar to the cyanolipid found in the seed oil of *Cardiospermum hirsutum* [46, 50, 65]. The signals for the methine (cyanohydrin) proton and vinyl protons are shifted downfield with respect to those of acacipetalin; a similar effect was observed in comparisons of the two previously described cyanolipids. The methylene protons of the aglycone appear as a doublet (again closely resembling those of the corresponding cyanolipid) at 4·13 $\delta$. When the spectrum was rerun in $C_6D_6$, the apparent doublet was more clearly an AB pattern (4 peaks). Peaks corresponding to sugar protons were all similar to those of acacipetalin (anomeric protons at 4·44 and 4·35 $\delta$ ($J$ 7 Hz in both) respectively, indicating that cardiospermin is also a $\beta$-glycoside, and likely has the same configuration as acacipetalin and lotaustralin. The presence of a hydroxyl group in the aglycone was demonstrated by the proton integral values of the acetate prepared from cardiospermine [47].

### CYANOGENIC COMPOUNDS WITH CYCLOPENTENE RINGS

Another type of cyanogenic compound contains a cyclopentene ring. Compounds of this group are of uncertain biosynthetic origin, but it has been

suggested [16] that they are derived from L-2-cyclopentene-1-glycine, and "that if a group of plants has the quirk of producing this amino acid, the conversion to deidaclin and its additionally hydroxygenated congeners involves little change in enzymatic specificity from the widespread synthesis of linamarin and lotaustralin". However, Zilg and Conn[47] have recently shown that flax seedlings lack the ability to synthesize deidaclin from D-L-2-cyclopentene-1-glycine when the amino acid was administered to cut shoots of the seedlings. Members of this group are found in a number of species of the taxonomically related families Flacourtiaceae and Passifloraceae.

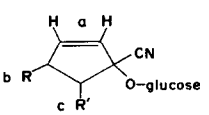
The IR spectra of all compounds of this type show peaks at 3200–3600 (*s*, hydroxyl) 2840–2960 (C–H stretch) and ~1000–1100 (*s*, ether stretching). Most other absorptions of deidaclin, tetraphyllin A, tetraphyllin B, barterin, and gynocardin are weak (gynocardin has a strong band at 1635 $cm^{-1}$).

The MS of deidaclin (70 eV, probe temperature 130°) had a peak at M-49 (as did barterin and gynocardin) and a pair of peaks at *m/e* 92 and 93 corresponding to the aglycone (R+) and (RH+). Barterin had a corresponding pair of peaks at 108, 109 and gynocardin had a single intense peak at 124 [16]. Tetraphyllin A and B have peaks corresponding to deidaclin and barterin respectively [30].

The vinyl protons of all compounds containing cyclopentene rings appear as two doublets, except in gynocardin where they are a singlet (Table 13). In the NMR spectra of the TMS ethers run in $CCl_4$, the two doublets were separated by 13 Hz (deidaclin), 33 Hz (tetraphyllin A), and 47 Hz when run in $D_2O$ [16]. Each was an AB multiplet appearing to be an unresolved triplet, at 6·00 and 6·40 $\delta$ respectively ($J$ 6 Hz). Those of barterin in $D_2O$ occurred at 6·05 and 6·30 $\delta$ (a separation of 25 Hz) [14]. The ring protons of the TMS ether of deidaclin occur at 2·47 $\delta$ (multiplet), tetraphyllin A at 2·46 $\delta$ (singlet), and those of deidaclin (in $D_2O$) occur at 2·6 $\delta$ [16]. In tetraphyllin B (Fig. 5, TMS ether in $CCl_4$) the ring protons, one of which is on a carbon bearing a hydroxyl group, are at 4·92 $\delta$ (distorted triplet) and appear as an ABX pattern, i.e. 2 quartets centered at 2·83 and 2·15 respectively. In the spectrum of barterin ($D_2O$) the two methylene protons appear as two doublets at 2·50 and 2·75 $\delta$ [14], and may possibly be the upper half of the tetraphyllin B set of quartets? These protons

Table 13. NMR data for cyanogenic compounds with cyclopentene rings

| | Signal from protons* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1a | 2a | 3a | 4a | 1b | 2b | 3b | 4b | 1c | 2c | 3c | 4c |
| Chemical shift ($\delta$) | 6·06<br>5·94 | 6·17<br>5·84 | 6·12<br>5·92 | 5·88 | 2·47 | 2·46 | 4·92 | 4·45 | 2·47 | 2·46 | †2·83<br>2·15 | 4·08 |
| Integral value | 2 | 2 | 2 | 2 | +1c<br>4 | +2c<br>4 | 1 | 1 | +1b<br>4 | +2b<br>4 | 2 | 1 |
| Multiplicity | 2<br>*d* | 2<br>*d* | 2<br>*d* | *s* | *m* | *s* | dis-<br>tort-<br>ed | AB<br>*m* | *m* | *s* | ABX<br>(8 pks) | *d* |
| Coupling constant (Hz) | ~6·0 | 6·0 | 6·0 | — | — | — | — | 5·0 | — | — | — | 5·0 |
| | 1d | 2d | 3d | 4d | 1e | 2e | 3e | 4e | 1f | 2f | 3f | 4f |
| Chemical shift ($\delta$) | 4·52 | ·4·46 | 4·44 | 4·42 | 3·68 | 3·68 | 3·71 | 3·73 | 3·0–<br>3·6 | 3·0–<br>3·7 | 3·0–<br>3·7 | 3·0–<br>3·7 |
| Integral value | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 4 | 4 | 4 | 4 |
| Multiplicity | *d* | *d* | *d* | *d* | *d* | *m* | *s* | *m* | complex pattern | | | |
| Coupling constant (Hz) | 7·0 | 6·5 | 7·0 | 7·0 | ~3·0 | — | — | — | — | — | — | — |

* Protons shown in formula 

: 1. deidaclin R = R′ = H; 2. tetraphyllin A R = R′ = H; 3. tetraphyllin BR = OTMS, R′ = H; 4. gynocardin R = R′ = OTMS; d. anomeric proton of sugar; e. $-CH_2OTMS$ of sugar; f. other sugar protons.

† 2 quartets centered at these $\delta$ values.

Table 14. Enzymes and other reagents that effect hydrolysis of cyanogenic glycosides

| Compound | Enzyme | Reference | Other reagents | Products | References |
|---|---|---|---|---|---|
| Acacipetalin | Almond emulsin | [13, 50, 72] | $Ba(OH)_2$ followed by acid |  | [13] |
| | linamarase | [37] | | | |
| Amygdalin | Almond emulsin | [9, 87] | Alkaline hydrolysis | Amygdalinic acid | [87] |
| | | | Acid | Mandelic acid | [96] |
| | | | Dilute mineral acid | HCN, benzaldehyde and glucose (2) | [97] |
| Barterin | Almond emulsin | [14] | 1 N $H_2SO_4$ | Glucose | [14] |
| Cycasin | Almond emulsin produces glucose and methylazoxy-methanol | [15, 26] | 1 N HCl | Glucose, $N_2$, $CH_2O$, MeOH | [26] |
| | Cycad emulsin | [15, 43] | Alkaline hydrolysis | Glucose, HCN, $N_2$ $HCO_2H$ | [26] |
| Deidaclin | Gynocardase | [9] | | | |
| | Linamarase | [9] | | | |
| Dhurrin | Almond emulsin | [17, 40, 41] | Hot dilute HCl | HCN, *p*-hydroxybenzaldehyde, glucose | [97] |
| | | | Alkaline conditions | Dhurrinic acid | [97] |
| *p*-Glucosyloxy-mandelonitrile | Spontaneously liberates HCN | [92] | | | |
| | Almond emulsin | [18] | | | |
| Gynocardin | Gynocardase | [9] | $Ba(OH)_2$ followed by Amberlite IR-120 (H+ form) | Gynocardinic acid acid | [19] |
| Holocalin | Emulsin | [20] | | | |
| Linamarin | Linamarase | [34, 69] | 0·2 N $Ba(OH)_2$ | α-Hydroxyisobutyric acid-β-D-glucose | [89] |
| | Gynocardase | [9] | | | |
| Lotaustralin | Linamarase | [34, 69] | Aq. $Ba(OH)_2$ followed by dilute HCl | (R)-2-Hydroxy-2-methylbutyric acid | [21] |
| Lucumin | Emulsin | [23] | 4 N HCl for 30 min | Xylose, glucose benzaldehyde | [36] |
| | | | 0·4 N HCl for 30 min | Prunasin | [36] |
| Macrozamin | | | 1 N HCl | $N_2$, $CH_2O$, MeOH, glucose and xylose | [24, 25] |
| | | | dilute NaOH | $N_2$, HCN, $HCO_2H$ | [24, 25] |
| | | | 1 N $NH_4OH$ | Primeverose | [24] |
| Neocycasin A | Cycad emulsin gives glucose | [26] | 0·2 N $H_2SO_4$ | Cycasin and glucose | [26] |
| | | | Alkaline solution | HCN, $CH_2O$ | [26] |
| | | | HCl | 2 glucose | [26] |
| Proteacin | Emulsin | [18, 70] | | | |
| Prunasin | Emulsin | [9, 28, 29] | Acid | (−)-Mandelic acid | [93, 94] |
| | Gynocardase | [9] | Dilute mineral acids | HCN, benzaldehyde, glucose | [97] |
| | Linamarase | [9] | | | |
| Sambunigrin | Emulsin | [42] | Acid | (+)-Mandelic acid | [91] |
| Taxiphyllin | Emulsin | [17] | | | |
| Tetraphyllin A | Linamarase | [30] | 1 N $H_2SO_4$ for 2 hr | Glucose | [30] |
| Tetraphyllin B | Linamarase | [30] | 1 N $H_2SO_4$ for 2 hr | 4-Hydroxy-2-cyclopenten-1-one and glucose | [30] |
| | | | $Ba(OH)_2$ followed by Dowex 50 (H+ form) | Tetraphyllinic acid | [30] |
| Triglochinin | Emulsin | [31, 91] | Acid | Triglochinic acid, glucose and HCN | [31] |
| | Gynocardase | [91] | | | |
| | Linamarase | [91] | | | |
| Triglochinin, methyl ester | Emulsin | [49] | | | |

*continued—*

Table 14 (contd.)

| Compound | Enzyme | Reference | Other reagents | Products | References |
|---|---|---|---|---|---|
| Vicianin | Emulsin | [32] | Acid | HCN | [32] |
| | Enzyme from *Vicia angustifolia* seeds gives the disaccharide vicianose, benzaldehyde and HCN | [95] | Conc HCl | Mandelic acid arabinose, glucose | [32] |
| Zierin | Emulsin | [33] | Sat $Ba(OH)_2$ sol followed by dil. HCl | *m*-Hydroxymandelic acid | [33] |

in the TMS ether of gynocardin are both on carbons bearing hydroxyls and are found at 4·45 and 4·08 $\delta$ as a pair of AB multiplets. The chemical shifts and coupling constants of the anomeric protons of TMS ethers of deidaclin, gynocardin, and tetraphyllin A and B are all at 4·42–4·52 $\delta$ with $J$ 7·0 Hz. Those in $D_2O$ are at ~4·85 $\delta$ [14, 16, 19]. Other sugar protons of TMS ethers of these glycosides occur between 3·0 and 3·7 $\delta$.

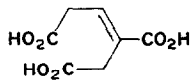
The absolute configuration of gynocardin has been established by X-ray crystallographic structure determination [66]. The C-1 position was previously known to have the D-configuration; it was established that the C-1 cyano group is *cis* to the hydroxyl at C-2 and *trans* to that at C-3. The stereochemical similarity of gynocardin and chaulmoogric acid suggests that deidaclin and barterin possess similar stereochemistry [16]. The similarity of NMR spectra of all these compounds confirms their structural similarity. It has been suggested [67] that barterin and tetraphyllin B are identical. Although we have not obtained an authentic sample of barterin, the literature values and our spectral studies of tetraphyllin B tend to confirm this view.

## CHARACTERIZATION AND SYNTHESIS

The aglycones of many cyanogenic compounds have been isolated and studied. This has often been accomplished by hydrolysis and concomitant derivatization with various reagents such as 2,4-dinitrophyenylhydrazine and semicarbazone. Most of the aforementioned compounds are cleaved by almond $\beta$-glucosidase at neutral pH, although some (linamarin, lotaustralin, and cyclopentene ring containing compounds) react slowly with this enzyme. These instead are sensitive to linamarase [68, 69] or gynocardase [9] preparations. Enzymes involved in the hydrolysis of cyanogenic glycosides have been summarized in Table 14. Most "enzymes" have proven to be mixtures of enzymes with differing activities upon closer investigation and none are absolutely specific [99]. For example, although amygdalin is not hydrolyzed at an appreciable rate with linamarase, prunasin is hydrolyzed slowly [69]. One component of linamarase isolated from flax can hydrolyze compounds with an aromatic nucleus. An enzyme isolated from sorghum [86] hydrolyzes dhurrin, taxiphyllin and prunasin. Some compounds such as acacipetalin and cardiospermin are hydrolyzed by both emulsin or linamarase [13, 37, 46, 50, 72]. Studies on the types and specificity of enzymes involved in the hydrolysis and dissociation of cyanogenic glycosides have been reviewed [4, 9, 10, 69, 87, 90].

With dilute mineral acid (HCl, $H_2SO_4$) or reagents that apparently give rise to these ($CHCl_3$) most cyanogenic compounds are hydrolyzed sufficiently to give a positive test for HCN. Several compounds (e.g. linamarin, lotaustralin, and dhurrin) give more rapid tests with dilute base (NaOH, KOH) [50, 88]. Treatment with stronger acid (e.g., 1N HCl or 1N $H_2SO_4$) usually produces the corresponding hydroxy-acid, frequently without racemization [10, 91, 92]. [e.g. *R*-prunasin gives *R*-mandelic acid]. Treatment with strong base usually produces the corresponding glycosidic acid but may also produce racemization [10, 87].

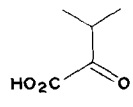
After hydrolysis, the resulting carbonyl compounds may be isolated by distillation, chromatography or derivatization. Compounds derived from tyrosine yield *p*-hydroxybenzaldehyde, which has been detected by TLC, PC (visualized by spray with 0·1% 2,4-dinitrophenylhydrazine in 2N HCl), UV spectroscopy [18, 41, 70], melting and mixed melting points [17], and preparation of the 2,4-dinitrophenylhydrazone (2,4-DNP) [27]. Benzaldehyde, from the series of compounds derived from phenylalanine, has been determined by preparation of the 2,4-DNP derivative [22, 29, 32, 36]. Triglochinin is an enol ether, and as such undergoes ready acid hydrolysis. The cyanohydrin can then lose HCN to yield a ketene which reacts with water to produce triglochinic acid [31, 58]. The structure of this acid (**30**) has been confirmed by synthesis [71].




(**30**) Triglochinic acid

*m*-Hydroxybenzaldehyde from zierin and holocalin has been identified by preparation of the phenylhydrazone, semicarbazone, and other derivatives [33]; by UV and IR spectroscopy; and by TLC on Kieselgel [20]. Linamarin and lotaustralin yield acetone and 2-butanone respectively. Mixtures of the 2,4-DNPs of these ketones have been separated on silica gel 1B-F TLC plates [42].

Steyn and Rimington reported the isolation of isobutyrylformic acid (α-ketoisovaleric acid) from the hydrolysis mixture of acacipetalin [13, 72]. We have recently shown that enzymatic hydrolysis of a mixture of glycosides from *Acacia sieberiana*, (Leguminosae) subsequent distillation, preparation of the 2,4-DNP (and as indicated by NMR spectroscopy) yields the 2,4-DNP of α-methylacrolein [50]. One can explain the origin of isobutyrylformic acid in Steyn and Rimington's materials by a shift of the double bond and hydrolysis of the enol ether and ketonization.




(**31**) Isobutyrylformic acid

The aglycone of cardiospermin, by analogy to that of the cyanogenic lipids of *Cordia verbenacea* (Boraginaceae) [50, 65], is unstable and we have been unable to isolate it or its derivatives. Compounds with cyclopentene rings have been hydrolyzed to yield the corresponding cyclopentenones. Deidaclin was hydrolyzed with gynocardase and the 2,4-DNP of 2-cyclopentenone prepared [16]. Russell and Reay, however, were unable to obtain a 2,4-DNP derivative of tetraphyllin A which has been considered identical to deidaclin by acid hydrolysis in (1 N $H_2SO_4$) [30]. The hydrolysate of tetraphyllin B, on the hand, readily yielded a 2,4-DNP derivative. 4-Hydroxycyclopent-2-eneone was identified by NMR spectroscopy, its optical rotation ([α]D 25–28·2°), UV spectroscopy (213 nm, $\epsilon = 6200$, EtOH) and its IR spectrum [30]. Paris *et al.* did not isolate an aldehyde from the hydrolysis mixture of barterin [14]. As the aglycone moiety apparently underwent spontaneous decomposition upon hydrolysis, Coburn and Long prepared gynocardinic acid by basic hydrolysis of gynocardin with barium hydroxide. They were able to convert this to methyl 1,2,3-trihydroxycyclopentane-1-carboxylate, which was characterized by NMR spectroscopy.

Pseudocyanogenic compounds yield formaldehyde on cleavage with β-glucosidase. Formaldehyde from cycasin and other pseudocyanogenic compounds has been determined by the chromotropic acid method [43].

Sugars in hydrolysis mixtures have largely been determined by standard TLC and PC techniques [52, 73, 75]. GLC has also proven useful for the identification of sugars in hydrolysis mixtures [75–77]. Glucose in holocalin [20], tetraphyllin A, tetraphyllin B [30], triglochinin [31], and barterin [14] has been confirmed by the highly specific glucose oxidase method. The number of sugar molecules in proteacin was determined by the glucose oxidase reaction followed by UV spectrophotometric determination of peroxide [18]. The sugars of vicianin were isolated from the hydrolysis mixture and identified by PC, by preparation of phenylosazones and measurement of the specific rotation. Additionally, the glycoside was methylated, hydrolyzed and 2,3,4-tri-*O*-Me-D-glucopyranose and 2,3,4-tri-*O*-Me-L-arabinopyranose isolated [32]. Eyjólfsson[36] demonstrated that partial hydrolysis of lucumin yielded prunasin (and thus has glucose adjacent to the aromatic system) but complete hydrolysis gave xylose and glucose. The

Table 15. Syntheses of cyanogenic glycosides

| Compound | Method | Reference |
|---|---|---|
| Sambunigrin and prunasin | The first four compounds in this table were all synthesized by treating the appropriate hydroxy-carboxylic acid ethyl ester with the acetobromosaccharide in the | [78] |
| Linamarin | presence of silver oxide, conversion to the acetate of the cyanogenic glycoside | [79] |
| Amygdalin | | [80, 81] |
| Dhurrin and tetraphyllin tetraacetates | Treatment of the cyanohydrin of the acetate of *p*-hydroxybenzaldehyde with acetobromoglucose in the presence of $Hg(CN)_2$ in $MeNO_2$ | [82] |
| Vicianin acetate | Treatment of the 2,3,4-triacetyl-$\beta$-glucoside of (−)mandelonitrile with triacetyl $\alpha$-arabinosyl bromide in the presence of silver oxide, and subsequent dehydration with phosphoryl chloride | [83] |
| Linamarin (and isolinamarin) | Treatment of acetone cyanohydrin with acetobromoglucose in the presence of $Hg(CN)_2$ in $MeNO_2$. The acetate was deacetylated with sodium methoxide in absolute MeOH | [22] |
| Lotaustralin (and epilotaustralin) | Same as above except the cyanohydrin of 2-butanone used, deacetylated with barium methoxide | [21] |
| *p*-Glucosyloxymandelonitrile | Tetraacetyl-*p*-glucosyloxybenzaldehyde was deacetylated with ammonia in MeOH. The resulting *p*-glucosyloxybenzaldehyde was converted to the cyanohydrin with HCN | [18] |

identity of the sugars was again established by PC, methylation of the glycoside and hydrolysis.

Nishida and co-workers were able to show that partial hydrolysis of neocycasin A gives cycasin and glucose establishing that the glycoside contains only glucose as the sugar component. An additional spot on PC had an identical $R_f$ value to that of laminaribiose [26]. These workers then prepared the octaacetyl derivative m.p. 159–160° which agreed with literature values. Lythgoe and Riggs[24] in a similar manner established that the sugar of macrozamin is primeverose, 6-($\beta$-D-xyloside)-D-glucose. The structures of several cyanogenic glycosides have now been confirmed by independent synthesis (Table 15).

*Note*: persons interested in receiving a mimeographed set of NMR spectra of cyanogenic glycosides mentioned in this article should contact the author.

*Acknowledgements*– I wish to express my appreciation for support under Biomedical Research Funds (NIH UPS RR 07030), to Dr E. E. Conn for a summer appointment in his laboratory at the University of California, at Davis, to the Institute for Advanced Studies of the University of Illinois for a fellowship (Spring 1973) and to the Graduate Research Board for the funds necessary to purchase the IR spectrophotometer and the gas chromatograph used in this study. The MS data processing equipment employed in the present study was provided by NIH grants CA 11388 and 16864 from the National Cancer Institute and the National Institute of General Medical Sciences respectively. We would like to thank the Department of Chemistry, NMR Spectroscopy Laboratory and especially S. Silber for the determination of NMR spectra. I would like to thank Dr E. E. Conn, C. Butterfield, C. Eggerding and W. Kawahara for their assistance in the development of this problem. Samples of cyanogenic compounds and information regarding past work have been graciously provided by: R. Eyjólfsson (Reykjavik), R. Gmelin (Berlin), R. Hegnauer and H. W. L. Ruijgrok (Leiden), G. H. N. Towers (Vancouver), A. Kobayashi (Kagoshima), P. F. Reay (Palmerston North), R. C. Clapp (Natick Laboratory), V. Plouvier (Paris), L. M. Larsen (København), and E. E. Conn (Davis).

## REFERENCES

1. Kingsbury, J. M. (1964) *Poisonous Plants of the U.S. and Canada*, Prentice-Hall, Englewood Cliffs, N.J.
2. Montgomery, R. D. (1969) in *Toxic Constituents of Plant Food Stuffs* (Liener, I. E., ed.) Academic Press, New York.

3. Osuntokun, B. O. (1971) *Trans. Roy. Soc. Trop. Med. Hyg.* **65,** 454.
4. For a brief review, see E. E. Conn (1974) Biosynthesis of Cyanogenic Glycosides. *Biochem. Soc. Symp.* **38,** 277.
5. Conn, E. E. (1974) Cyanogenetic Glycosides, in *Toxicants Occurring Naturally in Foods*, 2nd ed., Committee on Food Protection, National Research Council, Washington, D.C.
6. Mirande, M. (1909) *C. R. Acad. Sci., Paris* **149,** 140.
7. Guilbault, G. G. and Cramer, D. N. (1966) *Analyt. Chem.* **38,** 834.
8. Feigl, F., Gentil, V. and Jungreis E. (1959) *Microchim. Acta.* **44,** 47.
9. Tantisewie, B., Ruijgrok, H. W. L. and Hegnauer, R. (1969) *Pharm. Weekblad* **104,** 1341.
10. Eyjólfsson, R. (1971) *Fort. der Chem. Org. Naturst.* **27,** 74.
11. Mikolajczak, K. L., Smith, C. R. Jr. and Tjarks, L. W. (1970) *Lipids* **5,** 812.
12. Seigler, D. S. (1974) *Phytochemistry* **13,** 841.
13. Rimington, C. (1935) *Onderst. J. Vet. Sci. and Anim. Ind.* **5,** 445.
14. Paris, M., Bouquet, A. and Paris, R. (1969) *C. R. Acad. Sci. Paris* **268,** 2804.
15. Wells, W. W., Yang, M. G., Bolzer, W. and Mickelsen, O. (1968) *Anal. Biochem.* **25,** 325.
16. Clapp, R. C., Ettlinger, M. G. and Long, L. Jr. (1970) *J. Am. Chem. Soc.* **92,** 637.
17. Towers, G. H. N., McInnes, A. G. and Neish, A. C. (1964) *Tetrahedron* **20,** 71.
18. Sharples, D. and Stoker, J. R. (1969) *Phytochemistry* **8,** 597.
19. Coburn, R. A. and Long, L. Jr. (1966) *J. Org. Chem.* **31,** 4312.
20. Gmelin, R., Schüler, M. and Bordas, E. (1973) *Phytochemistry* **12,** 457.
21. Bissett, F. H., Clapp, R. C., Coburn, R. A., Ettlinger, M. G. and Long, L. Jr. (1969) *Phytochemistry* **8,** 2235.
22. Clapp, R. C., Bissett, F. H., Coburn, R. A. and Long, L. Jr. (1966) *Phytochemistry* **5,** 1323.
23. Bachtez, M., Prieto, E. S. and Canales, A. M. (1948) *Ciencia* **9,** 200.
24. Lythgoe, B. and Riggs, N. V. (1949) *J. Chem. Soc.* 2716.
25. Langley, B. W., Lythgoe, B. and Riggs, N. V. (1951) *J. Chem. Soc.* 2309.
26. Nishida, K., Kobayashi, A., Nagahama, T. and Numata, T. (1959) *Bull. Agr. Chem. Soc. Japan* **23,** 460.
27. Private communication from R. L. Young to E. E. Conn (1968).
28. Kofod, H. and Eyjólfsson, R. (1966) *Tetrahedron Letters* 1289.
29. Ben-Yehoshua, S. and Conn, E. E. (1964) *Plant Physiol.* **39,** 331.
30. Russell, R. B. and Reay, P. F. (1971) *Phytochemistry* **10,** 1373.
31. Eyjólfsson, R. (1970) *Phytochemistry* **9,** 845.
32. Kofod, H. and Eyjólfsson, R. (1969) *Phytochemistry* **8,** 1509
33. Finnemore, H. and Cooper, J. M. (1936) *J. Proc. Roy. Soc. N.S. Wales* **70,** 175.
34. Butler, G. W. and Butler, B. G. (1960) *Nature* **187,** 780.
35. Nahrstedt, A. (1970) *Phytochemistry* **9,** 2085.
36. Eyjólfsson, R. (1971) *Acta. Chem. Scand.* **25,** 1888.
37. Butterfield, C., Seigler, D. S. and Conn, E. E. (1974) in preparation.
38. Silverstein, R. M. and Bassler, G. C. (1963) *Spectrometric Identification of Organic Compounds*, J. Wiley, New York.
39. Bellamy, L. J. (1956) *The Infrared Spectra of Complex Molecules*. p. 225, John Wiley, New York.
40. Reay, P. F. (1969) *Phytochemistry* **8,** 2259.
41. Reay, P. F. and Conn, E. E. (1970) *Phytochemistry* **9,** 1825.
42. Conn, E. E. and Secor, J. B. (1974) in preparation.
43. Matsumoto, H. and Strong, F. M. (1963) *Arch. Biochem. Biophys.* **101,** 299.
44. Nahrstedt, A. (1973) *Planta Med.* **24,** 83.
45. Unpublished data from the E. E. Conn laboratory.
46. Seigler, D. S., Eggerding, C. and Butterfield, C. (1974) *Phytochemistry* in press.
47. Zilg, H. and Conn, E. E. (1974) *J. Biol. Chem.* in press.
48. Hegnauer, R. and Ruijgrok, H. W. L. (1971) *Pharm. Weekblad* **106,** 263.
49. Sharples, D., Spring, M. S. and Stoker, J. R. (1972) *Phytochemistry* **11,** 3069.
50. Seigler, D. S., unpublished data.
51. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, Springer-Verlag, Berlin.
52. Seigler, D. S., Mikolajczak, K. L., Smith, C. R. Jr., Wolff, I. A. and Bates, R. B. (1970) *Chem. Phys. Lipids* **4,** 147.
53. Shriner, R. L., Fuson, R. C. and Curtin, D. Y. (1964) *The Systematic Identification of Organic Compounds*, John Wiley, New York.
54. Waller, G. (1972) *Biochemical Applications of Mass Spectroscopy*, Wiley-Interscience, N.Y.
55. Riggs, N. V. (1954) *Aust. J. Chem.* **7,** 123.
56. Palekar, R. S. and Dastur, D. K. (1965) *Nature* **206,** 1363.
57. van der Veen, J. M. (1963) *J. Org. Chem.* **28,** 564.
58. Ettlinger, M. and Eyjólfsson, R. (1972) *Chem. Comm.* 572.
59. Sharples, D., Spring, M. S. and Stoker, J. R. (1972) *Phytochemistry* **11,** 2999.
60. Nakanishi, K. (1962) *Infrared Absorption Spectroscopy*, Holden-Day, San Francisco.
61. Nahrstedt, A. (1973) *Phytochemistry* **12,** 2799.
62. (1962) *NMR Spectra Catalog*, Vol. I and II, Varian Associates, Palo Alto, Calif.
63. Chizov, O. S., Molodtsov, N. V. and Kochetkov, N. K. (1967) *Carbohyd. Res.* **4,** 273.
64. Seigler, D., Seaman, F. and Mabry, T. J. (1971) *Phytochemistry* **10,** 485.
65. Mikolajczak, K. L., Seigler, D. S., Smith, C. R. Jr., Wolff, I. A. and Bates, R. B. (1969) *Lipids*, **4,** 617.
66. Kim, H. S., Jeffrey, G. A., Panke, D., Clapp, R. C., Coburn, R. A. and Long, L. Jr. (1970) *Chem. Commun.* 381.
67. Private communication from P. F. Reay.
68. Coop, I. E. (1940) *N.Z.J. Sci. Tech.* **22B,** 71.
69. Butler, G. W., Bailey, R. W. and Kennedy, L. D. (1965) *Photochemistry* **4,** 369.
70. Young, R. L. and Hamilton, R. A. (1966) Proc. Sixth Annual Meeting of the Hawaii Macadamia Producers Assoc., 27.
71. Eyjólfsson, R. (1970) *Acta Chem. Scand.* **24,** 3075.
72. Steyn, D. G. and Rimington, C. (1935) *Onderst. J. Vet. Sci. and Anim. Ind.* **4,** 51.
73. Clark, J. M. Jr. (1964) *Experimental Biochemistry*, W. H. Freeman, San Francisco.
74. Dawson, R. M. C., Elliott, D. C., Elliott, W. H. and Jones, K. M. (1969) *Data for Biochemical Research*, 2nd ed., Oxford Univ. Press, Oxford.
75. Sweeley, C. C., Bentley, R., Makita, M. and Wells, W. W. (1963) *J. Am. Chem. Soc.* **85,** 2497.
76. Kagan, J. and Mabry, T. J. (1965) *Anal. Chem.* **37,** 288.
77. DeJongh, D. C., Radford, T., Hribar, J. D., Hanessian, S., Bieber, M., Dawson, G. and Sweeley, C. C. (1969) *J. Am. Chem. Soc.* **91,** 1728.
78. Fischer, E. and Bergmann, M. (1917) *Chem. Ber.* **50,** 1047.
79. Fischer, E. and Anger, G. (1919) *Chem. Ber.* **52,** 854.

80. Kuhn, R. and Sobotka, H. (1924) *Chem. Ber.* **57,** 1767.
81. Campbell, R. and Haworth, W. N. (1924) *J. Chem. Soc.* **125,** 1337.
82. Kofod, H. and Eyjólfsson, R. (1966) *Tetrahedron Letters*, 5349.
83. Chaudhury, D. N. and Robertson, A. (1949) *J. Chem. Soc.* 2054.
84. Bennett, W. D. and Tapper, B. A. (1968) *J. Chrom.* **34,** 428.
85. Nahrstedt, A. (1970) *J. Chromat.* **50,** 518.
86. Mao, C. and Anderson, L. (1967) *Phytochemistry* **6,** 473.
87. Conn, E. E. (1969) *Ag. and Food Chem.* **17,** 519.
88. Seigler, D. S. and Bloomfield, J. J. (1969) *Phytochemistry* **8,** 935.
89. Butler, G. W. and Conn, E. E. (1964) *J. Biol. Chem.* **239,** 1674.
90. Zilg, H., Tapper, B. A. and Conn, E. E. (1972) *J. Biol. Chem.* **247,** 2384.
91. Hegnauer, R. and Ruijgrok, H. W. L. (1971) *Phytochemistry* **10,** 2121.
92. Abrol, Y. P., Conn, E. E. and Stoker, J. (1966) *Phytochemistry* **5,** 1021.
93. Bourquelot, E. and Herissey, H. (1907) *Compt. Rend. Soc. Biol.* **62,** 828.
94. Fischer, E. (1895) *Chem. Ber.* **28,** 1508.
95. Bertrand, G. and Weisweiller, G. (1910) *C. R. Acad. Sci. Paris*, **151,** 884.
96. Dilleman, G. (1958) Composés Cyanogénétiques in *Handbuch der Pflanzenphysiologie* (W. Ruhland, ed.) Vol. 8, Springer-Verlag, Berlin.
97. Dunstan, W. (1906) Ann. Rept.-Brit. Assoc. Adv. Science, 145. The Chemical Aspects of Cyanogenesis in Plants.
98. Jensen, S. R. and Nielsen, B. J. (1973) *Acta Chem. Scand.* **27,** 2661.
99. Haisman, D. R. and Knight, D. J. (1967) *Biochem. J.* **103,** 528.

*Other pertinent literature and reviews*

Conn, E. E. and Butler, G. W. (1969) The Biosynthesis of Cyanogenic Glycosides and Other Simple Nitrogen Compounds, in *Perspectives in Phytochemistry* (Harborne, J. B. and Swain, T., eds.) Academic Press, London.

Gibbs R. D. (1963) History of Chemical Taxonomy, in *Chemical Plant Taxonomy* (Swain, T., ed.) Academic Press, London.

Gibbs, R. D. (1974) *Chemotaxonomy of Flowering Plants*, Vols. I–IV, McGill–Queen's University Press, Montreal.

Hegnauer, R. (1959) Tàxonomic Value of Cyanogenesis in Higher Plants, in *Recent Advances in Botany*, Vol. I, University of Toronto Press, Toronto.

Hegnauer, R. (1971) *Pharm. Acta Helv.* **46,** 585.

Hegnauer, R. (1970) *Chronache di Chimica*, No. 27, March.

Hegnauer, R. *Chemotaxonomie der Pflanzen*, Vols. I–VI, Birkhäuser Verlag, Basel.

Miller, L. P. (1973) Glycosides, in *Phytochemistry*, Chapter 11, Vol. I, Van Nostrand–Reinhold, N.Y.

Seifert, P. (1955) Blausäure-Verbindungen, in *Moderne Methoden der Pflanzenanalyze* (Paech, K. and Tracey, M. V., eds.). Vol. IV. Springer-Verlag, Berlin.

Tschiersch, B. (1967) *Pharmazie* **22,** 76.