Catalpol and Methylcatalpol: Naturally Occurring Glycosides in *Plantago* and *Buddleia* Species

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(Received 9 November 1964)

1. A glycoside of the aucubin type has been isolated in crystalline form from *Plantago* and *Buddleia* species, and has been shown to be identical with catalpol (Lunn, Edward & Edward, 1962). Catalpol has not been found in the free state before, but occurs as its *p*-hydroxybenzoyl ester, catalposide, in the genus *Catalpa*. 2. A second glycoside of this type has been obtained in crystalline form from *Buddleia*, and has been shown to be a mono-*O*-methyl derivative of catalpol, for which the name 'methylcatalpol' is proposed. 3. Both *Plantago* and *Buddleia* species are known to contain aucubin. The concentrations of this glycoside and catalpol are comparable in *Plantago*. In *Buddleia* methylcatalpol predominates somewhat over catalpol. Yields of the individual glycosides were about 0.1% of the fresh weight of the leaves. 4. Bobbitt, Spiggle, Mahboob, Philipsborn & Schmid (1962) have suggested structures for catalposide and catalpol based on chemical and physical evidence, in particular on n.m.r. spectra. Reappraisal of this evidence and additional measurements have now confirmed these structures and show that the *Buddleia* glycoside is the 6-*O*-methyl derivative of catalpol.

While seedlings of various species were being examined for their content of soluble sugars it was noticed that aqueous extracts of *Plantago lanceolata* (rib-grass, ribwort plantain) contained two substances that reacted on paper with the benzidine-trichloroacetic acid reagent (Bacon & Edelman, 1951) to give intensely coloured spots with strong fluorescence in ultraviolet light. One, having R_f 0.35–0.40 in butan-1-ol-acetic acid-water (Partidge, 1948; rhamnose taken as R_f 0.37) and giving a grey spot with pink fluorescence, was identified as aucubin (already known to be a constituent of *Plantago*; Bourdier, 1907). The other, which gave a salmon-pink colour with yellow fluorescence and had a somewhat smaller R_f (0.27–0.32), was obtained in crystalline form by partition chromatography on Celite (Lemieux, Bishop & Pelletier, 1956) and has been identified as catalpol (Lunn *et al.* 1962), a product of the alkaline hydrolysis of catalposide, the characteristic glycoside of the fruit of *Catalpa ovata* (Claassen, 1888).

There was no evidence that catalposide itself was present in *Plantago*. Spots with colour reactions similar to those of aucubin and catalpol were usually seen near the solvent front of chromato-

grams of extracts, but they were very faint in freshly gathered material. It seemed most likely that they were the corresponding aglycones.

A search for catalpol-like substances in plants of related Families (Scrophulariaceae, Lentibulariaceae) and in several species known to contain aucubin or related glycosides was at first unsuccessful. (In this connexion it was noticed, and confirmed by tests with an authentic sample, that asperuloside gives no reaction with the benzidine-trichloroacetic acid spray.) The fresh plant material examined included *Aucuba japonica* and *Garrya elliptica*, which both contained, as expected, large amounts of aucubin. However, extracts of the leaves of *Buddleia globosa* (another known source of aucubin; Trim & Hill, 1952) and of *Buddleia variabilis* contained two such substances, one apparently identical with catalpol, the other having R_f about 0.50, and hence running in front of aucubin. Each was obtained in crystalline form from *B. globosa* by chromatography on charcoal-Celite columns and the first was shown to be identical with catalpol.

The identification of catalpol was helped by a comparison of its infrared spectrum (D.M.S. no. 12902) with that of aucubin (D.M.S. no. 12901), to which it showed a marked similarity. The absence

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of a carbonyl grouping distinguished it from a number of known glycosides of the aucubin type. The elementary analysis was consistent with the presence of an additional oxygen atom, which would give a relatively small increase in molecular weight. This was confirmed by a somewhat lower glucose content, measured by glucose oxidase after enzymic hydrolysis by a *Plantago* extract. Lunn, Edward & Edward (1961) suggested that catalposide was the *p*-hydroxybenzoyl ester of a hydroxy derivative of aucubin. Hydrolysis of catalposide with 0.1N-barium hydroxide at room temperature had yielded a substance that they named 'catalpol'. Its infrared spectrum (Lunn *et al.* 1962) was apparently identical with that of our unknown compound, but other properties (m.p. 207.5–209°; $[\alpha]_D^{25} - 122^\circ$ in 90% ethanol) differed somewhat from our measurements (m.p. 203–205°; $[\alpha]_D^{25} - 104^\circ$ in 90% ethanol, -102° in methanol). Bobbitt *et al.* (1962) later gave m.p. 201–203° and $[\alpha]_D^{25} - 104^\circ$ in methanol for the product of catalposide hydrolysis by a basic resin, and the acetate prepared from our substance had m.p. $[\alpha]_D$ and infrared spectrum in good agreement with the 'heptaacetyl catalpol' of Lunn *et al.* (1962), which is actually a hexa-acetate (cf. Bobbitt *et al.* 1962).

Treatment with N-sodium hydroxide at 100°, which has no effect on aucubin (Trim & Hill, 1952), converted the plantain glycoside mainly into a substance of smaller R_F (0.12 at 21°). This was isolated by chromatography on charcoal-Celite and yielded a crystalline acetate that resembled the acetate of the alkaline-transformation product reported by Bobbitt *et al.* (1962) in having m.p. 132–133°. Its infrared spectrum had absorption maxima identical with those reported for 'hepta-acetyl isocatalpol' by Lunn *et al.* (1962), and in addition the band at 3470 cm.⁻¹ (hydroxyl) reported by Bobbitt *et al.* (1962).

Despite the discrepancies noted above there can be little doubt that the glycoside from *Plantago* and *Buddleia* is catalpol, which therefore is accumulated in considerable amounts in the unsubstituted form in these plants, along with the closely related aucubin.

In *Buddleia* species a third glycoside is present. This, although evidently related to both the others, seemed from its infrared spectrum (D.M.S. no. 12903) and its colour reaction with benzidine to be closer to catalpol. On heating in N-sodium hydroxide it was rapidly transformed, giving a product with smaller R_F (0.25), which also indicated a close similarity to catalpol. The replacement of one hydroxyl group in the latter by a methoxyl group would give an empirical formula of C₁₆H₂₄O₁₀, which requires C, 51.1; H, 6.4; OMe, 8.2 (Found C, 51.3; H, 6.5; OMe, 8.1%). This would explain the greater R_F value relative to catalpol. Further

confirmation of this relationship was obtained from n.m.r. measurements (see below), and by methylating both substances; in each case the same crystalline hexamethyl derivative was formed in good yield. Thus the third *Buddleia* glycoside is a mono-*O*-methyl derivative of catalpol, and might conveniently be named 'methylcatalpol'. Crystallographic evidence was consistent with this formulation. The liberation of glucose by the action of an emulsin preparation showed that the methyl group was located on the aglycone. It then seemed that the location of this methyl group could be settled by a consideration of the n.m.r. spectra of the glycosides and their derivatives.

The n.m.r. spectrum of methylcatalpol (in D₂O) was similar to that of catalpol in the same solvent, showing the vinyl proton as a quartet centred at 3.5 τ with coupling constants $J_1 = 6$ cyc./sec. and $J_2 = 2$ cyc./sec and signals from three protons in the 4.6–5.4 τ region. A sharp signal at 6.4 τ (three protons), not present in the spectrum of catalpol, is attributed to the *O*-methyl group.

From a consideration of the structure of catalpol [Bobbitt *et al.* (1962); (I) in Fig. 1] it should be possible to assign the methoxyl group to either C-6 or C-10 by comparing the spectra of the acetates of the two compounds in the 4.6–5.4 τ region, since protons on carbons bearing secondary, but not

Fig. 1. (a) Structural formula of aucubin. (b) Formulae based on those proposed by Bobbitt *et al.* (1962) for: (I) catalpol ($R = R' = H$); (II) catalpol hexa-acetate ($R = R' = CH_3 \cdot CO$); (III) catalposide ($R = H$, $R' = p\text{-HO} \cdot C_6H_4 \cdot CO$); (IV) catalposide hexa-acetate ($R = CH_3 \cdot CO$, $R' = p\text{-CH}_3 \cdot CO \cdot O \cdot C_6H_4 \cdot CO$); (V) methylcatalpol ($R = H$, $R' = CH_3$).

primary, acetylated hydroxyl groups usually appear in this region (Jackman, 1955). The acetyl derivative of methylcatalpol (in CDCl_3) is a penta-acetate (two acetate signals corresponding to 15 ± 1 protons based on the vinyl signal centred at 3.7τ) and has signals from seven protons in the $4.6\text{--}5.4\tau$ region. Catalpol hexa-acetate, however, has eight protons in this region, suggesting that the methyl group is at C-6.

However, each compound has one more proton in the $4.6\text{--}5.4\tau$ region than would be expected from structures based on (I). Careful measurements of the spectrum of catalposide acetate revealed that it also had eight protons in this region, and not seven as reported by Bobbitt *et al.* (1962). Both integrals were based on their respective vinyl proton signals and on the ratios of their $4.6\text{--}5.4\tau$ to $5.4\text{--}6.6\tau$ protons, which were clearly 8:5 and not the expected 7:6. These values did not appear to be consistent with the presence of only one secondary acylated hydroxyl group in the aglycones.

A possible explanation for the discrepancy (proposed by Professor von Philipsborn, personal communication) is that one of the C-10 protons [the C-10 protons are not equivalent in (I) because of the neighbouring asymmetric centre at C-8] has shifted from the $5.4\text{--}6.6\tau$ region in the glycosides to the $4.6\text{--}5.4\tau$ region in the acetates, after the introduction of several anisotropic acetyl groups. This has now been confirmed for methylcatalpol by spin-decoupling measurements.

The 60 Mcyc./sec. spectrum of methylcatalpol shows a typical four-line AB-pattern in the $5.4\text{--}6.6\tau$ region, centred at $\tau = 5.68$ and $\tau = 6.1$ with $J_{AB} = 13$ cyc./sec., assigned to the coupled C-10 protons. In that of the acetate only one of the doublets ($\tau = 6.02$) is easily seen; the other, having shifted to the $4.6\text{--}5.4\tau$ region, is partly obscured by other signals. The 100 Mcyc./sec. spectrum, however, resolves the multiplet at lower field and shows clearly the presence of the second doublet at $\tau = 5.2$ with $J = 13$ cyc./sec. Confirmation of this assignment was obtained by irradiating the sample in the region $\tau = 5.2$ (corresponding to the low-field C-10 proton), which caused collapse of the $\tau = 6.02$ doublet to a slightly broadened singlet. Hence the seven protons in the $4.6\text{--}5.4\tau$ region are accounted for, and the position of the methoxyl group on the secondary carbon atom C-6 is established.

The similarity of the spectra of catalpol acetate and catalposide acetate to that of methylcatalpol acetate indicates that their anomalous integrals may be explained in a similar fashion, and a full paper dealing with these substances is soon to be published by Professor von Philipsborn and his colleagues.

The presence of relatively large amounts of

aucubin, catalpol and methylcatalpol in *Buddleia* would make this genus a very suitable material for the study of their biosynthesis and interconversion.

There are many reports of the medicinal uses of the plantain (cf. Wren, 1932), including references to its use in wound-healing and as a diuretic. Kimura, Okuda & Takano (1963) have reported on the diuretic properties of the *Catalpa* glycosides. Investigations of this kind should be helped by the knowledge that catalpol can be obtained easily in large amounts from a common weed of temperate regions.

EXPERIMENTAL

Physical methods. The n.m.r. spectra were measured on a Varian A-60 instrument (Varian Associates, Palo Alto, Calif., U.S.A.) at the University of Aberdeen. Infrared spectra were measured in KBr disks with a Grubb-Parsons Double-Beam Spectrometer type S4 equipped with a NaCl prism. Optical rotations were measured in 2 dm. tubes with a sodium light source.

Plant material. *Plantago lanceolata* L. and *Plantago major* L. were collected on waste ground or grown from seed in the gardens of the Institute. One bush each of *Buddleia globosa* and *Buddleia variabilis* was examined. For most purposes only the leaves were taken and these could be kept at -15° or dried at 50° without appreciable decomposition of the glycosides. Even in plantain leaves that had been heated until they became brown under conditions similar to those of Dijkshoorn (1961) there was little decomposition of the two glycosides.

Isolation of glycosides from P. lanceolata. A typical extraction procedure was as follows.

Fresh leaves (6.5 kg.; 1.4 kg. of dry matter) were chopped into 10 l. of water at 95° containing sufficient CaCO_3 (about 200 g.) to prevent the extract from becoming acid. After vigorous stirring for 30 min. the suspension was filtered through cloth and the residue re-extracted for 30 min. with 7 l. of water at 95° . The combined filtrates were reduced in volume to 750 ml. and poured into 4 vol. of ethanol. The precipitate was washed and the filtrate plus washings were reduced in volume to 400 ml. (287 g. of dry matter).

Several procedures were tried for the isolation of the glycosides from this aqueous extract, including adsorption on active carbon (Ultrasorb S.C.120/240; British Carbo-Norit Union Ltd., Grays, Essex) and clarification with basic lead acetate, but eventually it was found that direct application of the extract to a column of moist Celite (Lemieux *et al.* 1956) and development with butan-1-ol saturated with water effected a considerable purification, and if the appropriate fractions of eluate from this column were applied to a second column both glycosides could be obtained in crystalline form and purified further by re-crystallization. Thus the aqueous extract from 9.1 kg. of leaves (500 ml., about 300 g. of dry matter) was applied to a column made from 1.5 kg. of Celite no. 535 (11.5 cm. diam. $\times$ 69 cm. high; packed in three sections, 500 g. each). Fractions containing mainly aucubin, the unknown glycoside and glucose were combined (46 g. of dry matter) and applied to a 900 g. Celite column (8 cm. diam $\times$ 69 cm. high; packed in five sections). Of the fractions of effluent (each 50 ml.) the first 153 contained no glycosides, the next 43 only

aucubin, 17 a mixture of aucubin and the unknown glycoside, and 25 only the unknown glycoside. From the latter, 8g. of crystalline material was obtained. Similar yields of crystalline aucubin were obtained.

Isolation of glycosides from B. globosa. Leaves (250g.) previously stored at -15° were chopped into boiling water, the pH remaining near neutrality. The whole was blended, again heated to boiling and filtered through cloth. Ethanol (2vol.) was added to the filtrate. The precipitate was removed by centrifuging and the supernatant fluid evaporated to small bulk. This was applied to a column of a mixture of 40g. of British Drug Houses Ltd. Activated Charcoal and 40g. of Celite no. 535 and eluted with a gradient of aqueous ethanol made by dropping ethanol into 1l. of 35% (v/v) ethanol, 5ml. fractions being collected. Fractions 69–80 contained the unknown glycoside with traces of aucubin and catalpol (see below).

Fractions 53–63, which contained catalpol, with smaller amounts of aucubin, the unknown glycoside and sugars (sucrose, oligosaccharide etc.), were combined and evaporated to dryness, the pH being controlled by additions of Bio-Deminrolit resin (The Permutit Co. Ltd.). The residue was applied to a 20g. Celite column, which was developed with aqueous butan-1-ol, 5ml. fractions being collected. Fractions 50–56 were combined, evaporated to small bulk and applied to a further 20g. Celite column. Fractions 61–80 from this column contained almost pure catalpol, which was recrystallized from water (75mg.) and identified by its infrared spectrum.

Enzymic hydrolysis. Dialysed aqueous homogenates of *P. lanceolata* contained a hydrolytic enzyme system or systems that attacked all three glycosides. In each case glucose was liberated and could be estimated by glucose oxidase (Huggett & Nixon, 1957). Analyses of the crystalline glycosides by this method gave a glucose content of 53% for aucubin (calc. 5.%) and 48% for catalpol (calc. 50%).

All three glycosides were also hydrolysed by the crop juice of the snail *Helix pomatia* and by various emulsin preparations.

Identification of aucubin. Aucubin has already been shown to be present in *Plantago* species (Bourdier, 1907). The crystalline material prepared from *P. lanceolata* had m.p. 182° , $[\alpha]_D^{25} -171.7^{\circ}$ (c 1.7 in water) and an infrared spectrum identical with that of an authentic specimen kindly provided by Dr A. R. Trim.

Pharmacological properties. Samples of aucubin and catalpol were supplied to Edinburgh Pharmaceutical Industries Ltd., who reported that tests of anti-bacterial and anti-inflammatory effects were negative.

Identification of catalpol

This glycoside was accompanied by aucubin in extracts of all the samples of *Plantago* spp. and *Buddleia* spp. examined. In butan-1-ol-acetic acid-water at 21° they had R_F values of about 0.25 and 0.35 respectively, calculated from R_F 0.37 for rhamnose. At higher temperatures the R_F of rhamnose in this solvent was unchanged, but those of the glycosides increased, values of 0.37–0.39 being found for aucubin with solvent travel of 30 cm. on Whatman no. 1 paper at 25° . Under these conditions the other glycoside had R_F 0.31–0.32, the value given for catalpol by Lunn *et al.* (1962).

Catalpol was prepared from a sample of catalposide kindly provided by Professor J. T. Edward: 1.25g. was dissolved in 40ml. of ethanol, and 100ml. of water and 24g. of De-Acidite FF (OH⁻ form) were added. The mixture was heated at 50° for 1hr. with occasional shaking; paper chromatograms indicated complete conversion into catalpol and small amounts of material of smaller R_F (possibly isocatalpol). The resin was removed by filtration and the filtrate evaporated to dryness (0.77g.). The material was recrystallized three times from methanol, but still contained some impurity and so was chromatographed on a 20g. Celite column and recrystallized from methanol, yielding 0.26g. of catalpol, m.p. $203-205^{\circ}$, $[\alpha]_D^{25} -102^{\circ}$ [c 0.98 in 90% (v/v) ethanol]. This material showed no depression of m.p. when mixed with the plantain glycoside. Additional confirmation of their identity was obtained from their infrared spectra and by the preparation of derivatives.

The glycoside from *Plantago* was acetylated at 25° with acetic anhydride in pyridine. After 24hr. hexane was added (cf. Lunn *et al.* 1962) and crystals appeared. These had properties identifying it with the 'heptaacetyl catalpol' of Lunn *et al.* (1962), namely m.p. 140° , $[\alpha]_D^{25} -88^{\circ}$ (c 1.46 in chloroform); the band maxima found in the infrared spectrum agreed well with those given by Lunn *et al.* (1962), apart from a reported shoulder at 956cm^{-1} (found at 965cm^{-1}) and a band reported at 699cm^{-1} (found at 706cm^{-1}).

The glycoside (200mg.) was heated in 5ml. of *n*-NaOH for 10min., cooled, neutralized with acetic acid and applied to a column (30cm. $\times$ 2.5cm.) of a mixture of 40g. of British Drug Houses Ltd. Activated Charcoal and 40g. of Celite no. 535. Elution with a gradient produced by dropping ethanol into 1l. of water removed almost the whole of the transformation product before the small residue of the starting material. The product (30mg.) did not crystallize. It was acetylated with acetic anhydride in pyridine, giving a crystalline product (23mg.), m.p. $132-133^{\circ}$, $[\alpha]_D -105^{\circ}$ (c 1.2 in chloroform). Bobbitt *et al.* (1962) give m.p. $131-133^{\circ}$ for the hepta-acetyl derivative of the substance produced by opening the epoxide ring. Lunn *et al.* (1962) give m.p. $127-128^{\circ}$, $[\alpha]_D -121^{\circ}$ (c 2.21 in chloroform) for their 'heptaacetyl isocatalpol', and an infrared spectrum in carbon tetrachloride similar to but not identical with that of our acetate.

Unknown glycoside from Buddleia

Fractions from the charcoal-Celite column were evaporated to dryness and the glycoside was recrystallized from water, giving 130mg. of material, m.p. $236-238^{\circ}$, $[\alpha]_D^{25} -122^{\circ}$ [c 1.64 in 90% (v/v) methanol]. This material lost the equivalent of 1mol. of water/mol. on drying over P_2O_5 at 15mm. Hg pressure for 4 days at 61° .

Crystallography. The transparent colourless crystals had different habits, depending on the solvent from which they crystallized. From water they formed plates parallel to (001) and elongated parallel to (010), whereas those from propan-2-ol were plates parallel to (010). The refractive indices were $\alpha = 1.533$, $\beta = 1.565$ and $\gamma = 1.569$, with strong negative birefringence. The optic axial plane was parallel to (010) and $2V$ about 40° .

Single-crystal Weissenberg X-ray-diffraction patterns showed the crystals to be monoclinic with $a = 7.56\text{\AA}$, $b = 9.69\text{\AA}$, $c = 12.07\text{\AA}$ and $\beta = 104^{\circ}$, and the space group $P2_1/m$.

A density determination gave 1.46 g./ml. and this, with the above dimensions, shows the molecular weight to be a submultiple of 748 (twice $C_{16}H_{24}O_{10} = 752$).

Methylation of catalpol. Catalpol (0.98 g.) was dissolved in a mixture of 3.0 ml. of dimethylformamide and 4.0 ml. of methyl iodide, treated with 5 g. of Ag_2O at 45° for 5 hr. and left overnight at 21°. The Ag_2O was removed by filtration and washed with dry acetone. Filtrate and washings were evaporated to dryness under reduced pressure, the last traces of solvent being removed by prolonged exhaustion (3 hr.) by a high-vacuum pump. The residue (1.74 g.) crystallized. Treatment with charcoal in hexane gave 0.85 g. of crystals that after further recrystallization yielded 0.44 g. of *hexamethylcatalpol*, m.p. 79°, $[\alpha]_D^{23.5} - 91^\circ$ (c 1.96 in chloroform) (Found: C, 57.1; H, 7.7; OMe, 40.6. $C_{21}H_{34}O_{10}$ requires C, 56.6; H, 7.6; OMe, 41.7%). That methylation was complete in one operation was confirmed by the infrared and n.m.r. spectra, which showed the absence of hydroxyl groups and the presence of six methyl groups/mol.

Methylation of Buddleia glycoside. The glycoside (55 mg.) was dissolved in 0.3 ml. of dimethylformamide and 0.4 ml. of methyl iodide, 0.5 g. of Ag_2O was added, and the mixture was kept for 8 hr. at 45° and overnight at 21°. The product was isolated as for hexamethylcatalpol, yielding 42 mg. with m.p. 79°, unchanged by admixture with that substance and having an identical infrared spectrum. The two substances were indistinguishable when examined by thin-layer chromatography on Kieselgel G (E. Merck A.-G., Darmstadt, Germany) with chloroform-ether (1:1, v/v) as solvent.

This work was done with the technical assistance of Mrs S. M. Lindsay. We are indebted to Mr A. H. Knight of the Department of Plant Physiology for providing the materials on which the original observations were made. We thank Dr A. R. Trim for very helpful discussions and for gifts

of aucubin and asperuloside; also Professor J. T. Edward for a generous sample of catalposide. We are grateful to Professor von Philipsborn of the University of Zürich for help in interpreting the n.m.r. data and for carrying out the crucial spin-decoupling measurements with his Varian HR-100 instrument. We thank Mr K. C. Reid for the biological tests and our colleague Mr W. A. Mitchell for the crystallographic data. The interest and encouragement of Professor Sir Edmund Hirst, F.R.S., did much to bring this research to a satisfactory conclusion.

REFERENCES

- Bacon, J. S. D. & Edelman, J. (1951). *Biochem. J.* **48**, 114.
Bobbitt, J. M., Spiggle, D. W., Mahboob, S., Philipsborn, W. von & Schmid, H. (1962). *Tetrahedron Lett.* **8**, 321.
Bourdier, L. (1907). *J. Pharm. Chim., Paris*, **26**, 254.
Claassen, E. (1888). *Amer. chem. J.* **10**, 228.
Dijkshoorn, W. (1961). *Jaarb. Inst. biol. scheik. Onderz. LandbGewass., Wageningen*, p. 99.
Huggett, A. St G. & Nixon, D. A. (1957). *Lancet*, **273**, 368.
Jackman, L. M. (1955). *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, p. 55. London: Pergamon Press Ltd.
Kimura, K., Okuda, T. & Takano, T. (1963). *J. pharm. Soc. Japan*, **83**, 635. Cited in *Chem. Abstr.* (1963). **59**, 13114f.
Lemieux, R. U., Bishop, C. T., & Pelletier, G. E. (1956). *Canad. J. Chem.* **34**, 1365.
Lunn, W. H., Edward, D. W. & Edward, J. T. (1961). *Chem. & Ind.* p. 1488.
Lunn, W. H., Edward, D. W. & Edward, J. T. (1962). *Canad. J. Chem.* **40**, 104.
Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
Trim, A. R. & Hill, R. (1952). *Biochem. J.* **50**, 310.
Wren, R. C. (1932). *Potter's Cyclopaedia of Botanical Drugs and Preparations*, p. 271. London: Potter and Clarke Ltd.