Ergosterol is widely distributed in plants. Although it has been found in several lichens (1), its isolation from *P. caperata* has not been reported. This compound also was identified by GC–mass spectrometry in the unsaponifiable matter of the same lichen species, along with several other sterols (3) presenting a common biogenetic relationship.

EXPERIMENTAL[1]

The air-dried lichen[2] (330 g) was extracted in a soxhlet apparatus with petroleum ether (bp 40–60°) until the solvent was colorless. By concentration of this extract under reduced pressure, an amorphous product was formed (2.5 g after washing with petroleum ether and drying). It showed, on TLC [Kieselgel G[3] plates activated at 110° for 30 min, thickness ~0.25 mm, developed with chloroform–acetone (80:20)], a spot corresponding to (+)-usnic acid, which was isolated and identified by melting point, $[\alpha]_D$, and UV, IR, and mass spectral data (1, 4, 5).

Further concentration of the petroleum ether extract to 50 ml gave another amorphous substance (675 mg), 105 mg of which was extracted by methanol. The resulting orange solution was submitted to TLC as described previously and showed spots corresponding to usnic acid, atranorin, and chloratranorin (?). It also showed a blue spot (R_f ~0.5), after spraying with anisic aldehyde (6), and a yellow spot (R_f ~0.4) in visible light, became red under UV (350 nm). On concentration and cooling of the methanolic solution, a small amount of orange rectangular plates was formed.

Recrystallization from methanol gave 5 mg of pinastric acid (corresponding to the yellow-red spot), mp 204–206°; UV: λ_{max} (methanol) 293 and 388 nm; λ_{min} 238 and 338 nm; IR: ν_{max} (potassium bromide) 3020, 2960, 2840, 2540, 1775, 1765, 1680, 1600, 1575, 1520, 1465, 1450, 1440, 1420, 1370, 1335, 1310, 1280, 1260, 1195, 1160, 1110, 1090, 1070, 1030, 965, 910, 850, 840, 820, 790, 775, 740, 710, and 700 cm^{-1}; mass spectrum: M^+ = 352 (24%), *m/e* (%) 353 (5), 321 (20), 320 (100), 308 (6), 294 (7), 293 (3), 292 (11), 278 (2), 265 (10), 264 (50), 237 (10), 234 (2), 209 (14), 208 (80), 175 (14), 165 (10), 164 (2), 149 (6), 148 (20), 147 (50), 146 (10), 145 (40), 135 (10), 120 (11), 119 (56), 118 (8), 117 (28), 105 (18), 91 (19), 90 (14), 89 (71), 77 (19), 76 (18), 65 (18), and 63 (18); m* 164 (264 → 208); m* 207.5 (264 → 234); m* 264 (293 → 278); m* 291 (352 → 320).

By concentration of mother liquors from which pinastric acid was separated, a small amount of colorless plates was obtained (7 mg). Recrystallization from methanol gave 5 mg of ergosterol[4], mp 159–161° (Liebermann positive). Only one spot (the blue one referred to previously) was obtained on TLC [chloroform–acetone (80:20) and benzene–dioxane–acetic acid (90:25:4)]; UV: λ_{max} (methanol) 271, 282, and 293 nm; IR: ν_{max} (potassium bromide) 3390, 2900, 2840, 1667, 1471, 1370, 1070, 1042, 990, 971, 835, and 800 cm^{-1}; mass spectrum: M^+ = 396 (100%), *m/e* (%) 397 (30), 337 (50), 271 (45), 253 (56), 251 (23), 211 (49), 199 (28), 197 (30), 185 (28), 183 (28), 158 (65), 157 (70), 145 (78), 143 (85), 119 (58), 109 (55), 107 (60), and 105 (75).

[1] Melting points were determined on a Kofler microscope and are uncorrected. UV spectra were determined on a Bausch & Lomb Spectronic 505 spectrophotometer. IR spectra were determined on a Perkin-Elmer 257 instrument, and only the major bands are quoted. Mass spectra were recorded on a Hitachi RMU-6M with an ionizing potential of 70 ev.

[2] The plant material was collected from *Pinus Pinaster* Sol. trees at Cortegaça, Portugal, in May 1972 and identified as *P. caperata* by Prof. C. Tavares. A voucher specimen is deposited in the Laboratório de Quimica Orgânica, Faculdade de Farmácia, Universidade do Porto, Portugal.

[3] Merck.

[4] All analytical determinations were compared with an authentic sample.

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Quantitative NMR Analysis of a Four-Component Mixture of Phenylglycine Derivatives

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Abstract □ A rapid, accurate, and precise NMR analytical method for the analysis of phenylglycine, dihydrophenylglycine, tetrahydrophenylglycine, and cyclohexylglycine in combination with each other was developed. The method is based on the integration of the NMR signal characteristic of each component relative to the signal from tetramethylammonium bromide, which is added as an internal standard. No prior separation of the four components is required.

Keyphrases □ Phenylglycine and derivatives—NMR analysis in four-component mixture □ NMR spectroscopy—analysis, phenylglycine and derivatives in four-component mixture

NMR spectroscopy is being used increasingly for quantitative analysis of pharmaceuticals in dosage forms (1, 2) and chemical (3, 4) and isomeric (5) mixtures. The method offers advantages of speed, relatively good precision and accuracy, and ease of execution, and four components do not need to be sepa-

Table I—Chemical Shifts and Integration of Signal Characteristic of Individual Components

Component	Spectrum Integrated, Hz	Chemical Shift, ppm	Number of Protons
Phenylglycine	1000	7.60	5
Dihydrophenylglycine	300	2.84	4
Tetrahydrophenylglycine	1000	1.92	5
Cyclohexylglycine	300	1.25	5
Tetramethylammonium bromide	1000, 300	3.36	12

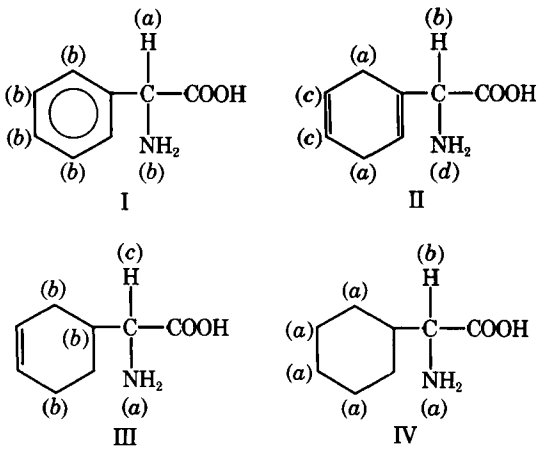
rated. The method described here is illustrative of the utility of NMR to determine complex mixtures of organic substances quantitatively without prior separation.

Catalytic reduction of dihydrophenylglycine usually results in a four-component mixture containing varying amounts of phenylglycine, tetrahydrophenylglycine, and cyclohexylglycine. In this laboratory, analysis of such mixtures have been carried out with an amino acid analyzer, a process that is much more time consuming than the NMR method presented here. The NMR method allows analysis of the bulk chemical with no prior separation and no treatment other than dissolving the sample in 2% DCl.

EXPERIMENTAL

Apparatus and Reagents—The following were used: a 90-MHz spectrometer[1], a five-place balance[2], a 2% DCl solution (prepared by diluting a stock solution[3] of 20% DCl 1 to 10 with deuterium oxide), tetramethylammonium bromide[4] (internal standard) (98% pure by titration), phenylglycine[4], tetrahydrophenylglycine[5], dihydrophenylglycine[5], and cyclohexylglycine[5].

Procedure—A sample (50–100 mg) containing dihydrophenylglycine as the major component and phenylglycine, tetrahydrophenylglycine, and cyclohexylglycine as minor components was accurately weighed into a small vial. A 10–20-mg quantity of tetramethylammonium bromide was accurately weighed and added to the vial. The resulting mixture was dissolved in 0.5 ml of 2% DCl solution. The NMR spectrum of the clear solution was obtained along with five integrations, using a 10-ppm sweep width and a 5-min sweep time. The portion of the spectrum from 4.0 to 0.5 ppm (δ scale) was rerun on a 300-Hz sweep width and a 5-min sweep time. This portion of the spectrum was integrated five times.



[1] Perkin-Elmer model R32.
[2] Model H16, Mettler.
[3] Thompson-Packard.
[4] Eastman Chemical Co.
[5] Prepared and assayed in these laboratories.

Table II—Analysis of Prepared Mixtures

Mixture	Components in Mixture	Weight Added, mg	Weight of Component Found by NMR	Percent Theory Recovered
1	Dihydrophenylglycine	66.93	66.34	99.1
	Phenylglycine	13.84	14.11	101.9
	Cyclohexylglycine	7.75	7.66	98.4
	Tetrahydrophenylglycine	6.91	7.32	105.9
	Tetramethylammonium bromide	11.59		
2	Dihydrophenylglycine	63.88	65.39	102.4
	Phenylglycine	15.71	15.05	95.8
	Cyclohexylglycine	10.13	10.17	100.4
	Tetrahydrophenylglycine	9.75	9.04	92.7
	Tetramethylammonium bromide	11.92		
3	Dihydrophenylglycine	48.74	49.42	101.4
	Phenylglycine	20.68	21.09	101.9
	Cyclohexylglycine	15.71	15.34	97.9
	Tetrahydrophenylglycine	12.02	11.30	94.0
	Tetramethylammonium bromide	14.08		
4	Dihydrophenylglycine	47.16	46.37	98.3
	Phenylglycine	9.76	9.46	96.9
	Cyclohexylglycine	13.06	13.75	105.3
	Tetrahydrophenylglycine	6.03	6.43	106.6
	Tetramethylammonium bromide	13.59		

The signal integrations used for calculating the individual components are summarized in Table I.

Each component in the sample was calculated using the average of five integrations according to:

$$\frac{\text{integral average}_{\text{sample}}}{\text{integral average}_{\text{TMAB}}} \times \frac{\text{equivalent weight}_{\text{sample}}}{\text{equivalent weight}_{\text{TMAB}}} \times \text{mg}_{\text{TMAB}} = \text{mg of component in mixture} \quad \text{(Eq. 1)}$$

where TMAB = tetramethylammonium bromide, and:

$$\text{equivalent weight} = \frac{\text{molecular weight}}{\text{number of protons integrated}} \quad \text{(Eq. 2)}$$

Equivalent weights are as follows: phenylglycine, 30.24; dihydrophenylglycine, 38.30; tetrahydrophenylglycine, 38.55; cyclohexylglycine, 31.24; and tetramethylammonium bromide, 12.84.

Tetrahydrophenylglycine is found by difference, *i.e.*, total weight of sample − (milligrams of phenylglycine + milligrams of dihydrophenylglycine + milligrams of cyclohexylglycine) = milligrams of tetrahydrophenylglycine.

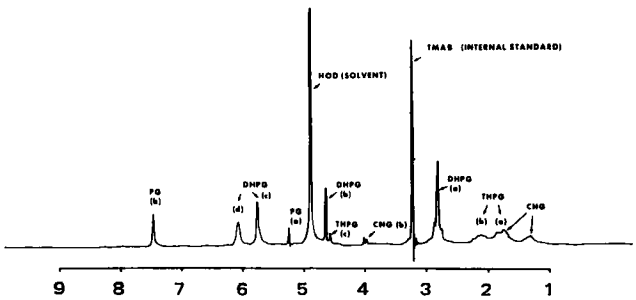


Figure 1—*NMR spectrum of four-component mixture of dihydrophenylglycine (DHPG), phenylglycine (PG), cyclohexylglycine (CHG), tetrahydrophenylglycine (THPG), and the internal standard tetramethylammonium bromide (TMAB).*

Table III—Statistical Analysis: Absolute Error and Percent Deviation from Average

Dihydrophenylglycine	Phenylglycine	Cyclohexylglycine	Tetrahydrophenylglycine
99.1	101.9	98.4	105.9
102.4	95.8	100.4	92.7
101.4	101.9	97.6	94.0
98.3	96.9	105.3	106.6
$\bar{x}$ = 100.3	99.1	100.4	99.8
σ = ±1.92	±3.24	±3.46	±7.47
RSD, % = 1.91	3.27	3.45	7.48

RESULTS AND DISCUSSION

The NMR spectrum of a four-component mixture with dihydrophenylglycine (II) as the major component and phenylglycine (I), tetrahydrophenylglycine (III), and cyclohexylglycine (IV) as minor components is shown in Fig. 1. Tetramethylammonium bromide was chosen as the internal standard because it is readily available and gives a single NMR signal at 3.36 ppm; this signal does not interfere with the NMR spectra of the four components.

The NMR signal characteristic of each component is indicated in Fig. 1 and listed in Table I. Once these characteristic NMR signals have been established and assigned, the method is straightforward. The remaining operation is to integrate the respective signals carefully relative to the signal of a known weight of the internal standard. These data are sufficient to calculate the amount of each component present in the mixture by Eqs. 1 and 2.

The results for a series of four-component mixtures prepared for analysis by the NMR method are shown in Table II. Since two components are present in amounts less than 10%, the accuracy and precision of this complex mixture are reasonably adequate (Table III). The tetrahydrophenylglycine content is found by difference due to the overlap of this signal with part of the NMR signal from cyclohexylglycine.

The developed method has the advantage over existing methods in that no prior separation of the components is required. An additional advantage is that qualitative identification of the individual components is obtained from their NMR spectra.

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Electron-Capture GLC Determination of Phenylpropanolamine as a Pentafluorophenyloxazolidine Derivative

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Abstract □ A simplified procedure is described for an electron-capture GLC determination of phenylpropanolamine in blood plasma. The method is based on derivatization of phenylpropanolamine with pentafluorobenzaldehyde at room temperature without prior extraction of the drug from plasma. The derivative, pentafluorophenyloxazolidine, is readily extracted from plasma into a small volume of hexane. Samples usually can be injected directly into the gas chromatograph without concentration of the hexane solution. Data are presented to illustrate the suitability of the method for dosage form bioavailability evaluation from plasma phenylpropanolamine levels achieved after acute oral administration of a typical dosage form.

Keyphrases □ Phenylpropanolamine—GLC analysis, plasma □ GLC—analysis, phenylpropanolamine, plasma □ Adrenergic agents—phenylpropanolamine, plasma

Previously reported methods for GLC determination of phenylalkanolamines and related compounds in plasma are based upon extraction of the amine from plasma followed by conversion to an electron-capturing perfluoroacyl- or pentafluorobenzaldehyde derivative (1–10). While this approach provides adequate sensitivity, the extraction procedure necessary to isolate the relatively water-soluble phenylalkanolamine from plasma sometimes gives rise to troublesome interfering peaks in the chromatogram.

A marked improvement in assay simplicity, together with excellent sensitivity and reliability, can be achieved by derivatization of phenylpropanolamine directly in plasma. The principle of the method involves reaction of the phenylpropanolamine in plasma with pentafluorobenzaldehyde to yield an electron-capturing derivative, which is then readily extracted into a small volume of hexane. The derivative forms within 1 hr at room temperature and is extremely stable under the assay conditions.

EXPERIMENTAL

Chromatographic Conditions—The gas chromatograph[1] was equipped with both an alkali flame-ionization detector and a 250-mCi tritium electron-capture detector. Glass columns, 2 m in length, 0.625 cm o.d., 0.20 cm i.d., were packed with 100–120-mesh

[1] Varian Aerograph model 2700, Varian Instrument Division, Palo Alto, Calif.