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DETERMINATION OF ALLANTOIN IN *PROTEA* SEED

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Abstract—Quantitative measurement of the downfield N_3–H proton in the PMR spectrum of allantoin provides a rapid and accurate method for its estimation in plant material. This method has been used to determine allantoin in the seed of *Proteaceae* species.

Racemic allantoin (**1**) has recently been isolated from the seed of *Protea compacta* [1]. This compound is a common constituent of plants but its function and that of ureides in general remains a controversial subject [2]. It has been postulated that it serves as a storage product for nitrogen; is the form in which nitrogen is translocated in plants; or might be a product in the detoxification of ammonia in plant tissue [2, 3].

Examination of the PMR spectrum of allantoin revealed that the cyclic N_3–H proton with a carbonyl group on either side of it, shifts very far downfield to δ10·53. This is a region of the spectrum where very little interference from other protons occurs, a fact which renders this particular proton suitable for analytical purposes. Although the PMR spectrum of allantoin has been recorded earlier [4], to date, the possibility of using the characteristic properties of the downfield proton (δ10·53) for analysis has not received attention. Since it was of interest to determine the levels of allantoin in germinating *Protea compacta* seed, the feasibility of employing the PMR method for estimating allantoin was investigated.

RESULTS

Allantoin is insoluble in practically all organic solvents. It will dissolve in hot water but this solution cannot be used for PMR analysis as the allantoin rapidly crystallizes out on cooling. It does, however, dissolve readily in warm dimethylsulphoxide (DMSO) and remains in solution even at low temperatures. DMSO is well suited as a solvent for allantoin since all the protons of the latter lie well downfield of the methyl signals of the DMSO. This fact makes it unnecessary to use the expensive $(CD_3)_2SO$ as solvent.

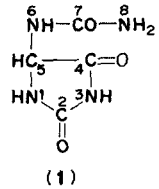
With the aid of synthetic allantoin, obtained by the oxidation of uric acid (**2**) [5], a standard curve was obtained relating the peak height of the downfield proton (δ10·53) to the concentration of allantoin. The weights of allantoin used for this graph ranged from 2–20 mg. This standard curve was used for the determination of allantoin in proteaceous seed.

Some difficulty was initially encountered in dissolving the dry plant extract in DMSO. This problem was overcome by adding maleic anhydride which facilitates dissolution of allantoin by the formation of the *bis*-allantoin derivative. Control tests, on pure allantoin in this solvent mixture, established that all protons except the two on the terminal amino group were unaffected. By following the PMR spectrum over a period of time (5 hr) it was found that the broad amino signal at δ5·77 gradually disappeared and was replaced by a sharp singlet, presumably –NH.CO.CH=CH.CO.NH–, further downfield. In order to obtain reproducible results freshly prepared DMSO containing maleic anhydride as an external standard was used in all determinations.

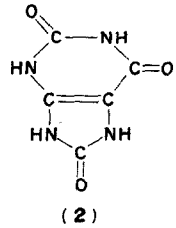
Estimation of allantoin concentrations in dry seed of *Protea compacta* gave a mean value of 2·04 ± 0·08%. In seed of *Protea neriifolia* and *P. repens* the allantoin content was 1·65 ± 0·07% and 1·12 ± 0·08% respectively. *Leucadendron daphnoides* and *Leucospermum reflexum* had no detectable allantoin.

DISCUSSION

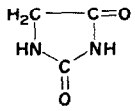
The most commonly-used method for the estimation of allantoin involves its degradation to urea and glyoxylic acid. The concentration of the latter substance is then determined colorimetrically [6] or spectrophotometrically [7] as the phenylhydrazone. A detailed evaluation of this method was undertaken by Young and Conway in 1942 [8]. The method is still widely-used today but certain modifications to eliminate secondary reactions by interfering substances have been incorporated [9, 10]. Another method employs the conversion of allantoin to the yellow *p*-dimethylaminobenzylideneureidohydantoin followed by its spectrophotometric estimation [11]. This technique has the drawback that allantoin must first be separated (by chromatographic techniques) from other compounds that are also capable of giving a positive reaction.



(1)



(2)



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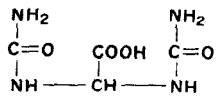

(4)

The PMR method cannot compete with the spectrophotometric techniques when only fractions of a mg of sample are available. Between 15–50 mg of plant extract is required and this should contain at least 1·5 mg of allantoin. The method does, however, have certain distinct advantages. The analysis is on the *intact* molecule and the method is *specific* for compounds bearing a hydantoin ring. Hydantoin (**3**) itself is unlikely to cause complications as its occurrence in plants is extremely rare [2]. It is of interest to note that the N_3–H proton of hydantoin resonates at δ10·70 [12]. Uric acid (**2**) which lacks a hydantoin ring but which commonly occurs with allantoin, will not interfere because the corresponding downfield proton is likely to have a different chemical shift and also because it is insoluble in the solvents used. Uric acid is practically insoluble in hot water, and in DMSO only a 22% solution was obtained under the present experimental conditions. Allantoic acid (**4**), which is commonly found with allantoin, and which interferes with the standard estimation of the latter since it also gives glyoxylic acid on degradation, does not possess the corresponding downfield proton. Organic acids, aldehydes and oximes, all of which possess protons resonating far downfield, and which are likely to be present in crude plant extracts, will not interfere since their protons have different chemical shifts. Sugars, lipids and amino acids were therefore not expected to affect the estimation of allantoin.

The method described should prove useful in cases where a rapid screening of plant extracts, known to contain significant amounts (>5%) of allantoin, is required. Allantoin levels of this magnitude are not uncommon [2].

The main food reserve in *Protea* species is protein (±60%) whereas in *Leucadendron daphnoides* and *Leucospermum reflexum* (genera in the same family), it is lipid (±50%). This observation, coupled with the analytical results, suggests that allantoin functions as a storage product for nitrogen in *Protea* species.

EXPERIMENTAL

PMR estimations were carried out on a 60 M Hz instrument. Pure allantoin from *P. compacta*, mp 235°, had the following PMR spectrum (DMSO): δ 10·53 (*s*, 1H, –CO–NH–CO–), 8·05 (*s*, –CH–NH–CO–), 6·90 (*d*, 1H, –CH–NH–CONH$_2$), 5·77 (*s*, 2H, –CONH$_2$), 5·26 (*q*, 1H, –NH–CH–NH–).

Method of estimation. Dried embyro material (1 g) was finely ground and extracted with boiling H_2O for 15 min. The hot soln was filtered at the pump and concentrated to dryness at 50° *in vac*. Between 15 and 50 mg of dried extract was then dissolved in 0·4 ml of freshly prepared DMSO containing maleic anhydride (250 mg/10 ml). To facilitate soln the mixture in the PMR tube was heated at 100° for 3 min. On occasions the soln turned brown. It was also usual for a small fraction to remain undissolved. The average height (5 consecutive scans) of the proton at δ 10·53 was then measured and the amount of allantoin calc. from the standard curve. In none of the plant extracts

were any interfering signals found further downfield than δ 8·50.

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