Phytochemistry, Vol. 20, No. 8, pp. 2009–2016, 1981.
Printed in Great Britain.

0031-9422/81/082009-08 $02.00/0

RADIOIMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF SCOPOLAMINE*

ELMAR W. WEILER, JOACHIM STÖCKIGT and MEINHART H. ZENK

Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum, D 4630 Bochum, West Germany

(*Revised received* 12 *December* 1980)

Key Word Index—*Datura*; *Duboisia*; Solanaceae; radioimmunoassay; scopolamine.

Abstract—A radioimmunoassay for the determination of pmol amounts of the tropane alkaloid scopolamine has been developed. The assay uses tritiated [*N*-C^3H_3]scopolamine of high specific activity (0.67 Ci/mmol) as tracer. The measuring range of the assay extends from 0.5 to 50 ng of scopolamine, and as little as 200 pg may be detected. The antiserum raised against a conjugate of scopolamine-*N*-β-propionic acid–human serum albumin is highly specific, and neither hyoscyamine, 6-hydroxyhyoscyamine, scopine, tropic acid nor other related alkaloids interfere in the scopolamine determination in crude plant extracts. This assay allows for the first time the rapid, sensitive and precise (CV = 2.5%) determination of this alkaloid in unpurified extracts of scopolamine-containing plants. The distribution of scopolamine in *Datura* plants, as well as its diurnal changes in leaf concentrations, has been investigated in detail and a preliminary survey on the variability of scopolamine leaf concentrations in a population of *Datura sanguinea* plants is given.

INTRODUCTION

The tropane alkaloid scopolamine is of considerable pharmaceutical interest because of its parasympatholytic, anti-cholinergic and anti-emetic as well as sedative action. The compound is extracted on an industrial scale from a variety of plant species such as members of the genus *Datura* and *Duboisia* [1]. Besides scopolamine, a range of related tropane alkaloids are found in the plant kingdom [2], the most abundant being hyoscyamine, which often occurs in excess of scopolamine. Hyoscyamine is regarded as the biosynthetic precursor of scopolamine via 6-hydroxyhyoscyamine [3]. Whereas the biosynthesis of scopolamine, its distribution within the plant and its seasonal variation have been studied [2–6], very little is known about the individual variability of the scopolamine content of plants. This knowledge, however, would be a prerequisite in breeding programs designed to select high-producing plant lines. This lack of information is mainly due to great seasonal and within-plant fluctuations in alkaloid content [6, 7] and because quantitative scopolamine determinations on a larger sample scale are still problematic.

Tropane alkaloids are currently quantitated by a number of methods including colorimetric determinations in purified extracts or after TLC separation of extracts [7, 8] and, more recently, by GLC [9–11]. The sensitivity of these methods is in the lower microgram range and only a few samples can be processed and analysed per day.

Radioimmunoassay (RIA) has proven very useful for the determination of plant constituents of diverse chemical structure [12–16] and offers the following advantages: very low concentrations of compounds may be quantitated precisely; the assay is usually applicable to the analysis of crude, unprocessed plant extracts, and it is readily mechanized. Thus, RIA is an efficient analytical method for large scale screening programs [16].

We report here on a highly specific and precise radioimmunoassay for nanogram quantities of scopolamine which considerably improves the analysis of this compound. The assay is applied to the analysis of scopolamine in individual plants of *Datura* species and its distribution within the organs of single plants. In addition, the daily course of alkaloid content was investigated in phytotron-grown plants. The assay reported here is not affected by the presence of excessive amounts of hyoscyamine and, due to its sensitivity and specificity, should also be useful in clinical studies on scopolamine.

RESULTS AND DISCUSSION

General assay parameters

The rabbits immunized with the human serum albumin (HSA) conjugate of glutaryl-(−)-scopolamine (Fig. 1) did not produce significant amounts of antibody. In contrast, all the rabbits immunized with the nor-(−)-scopolamine-*N*-β-propionic acid–HSA conjugate (Fig. 1) developed anti-scopolamine antibodies. However, from a large number of immunized animals only one produced a high-titred, and at the same time, very specific antiserum. This antiserum has been characterized and was used for the present study.

At a final assay dilution of 1:2250 (0.1 ml of 250-fold diluted antiserum per assay tube), this serum bound 30% of an added 23.7 pmol (35000 dpm, 5000 cpm) of [*N*-C^3H_3]scopolamine (sp. act. 0.67 Ci/mmol), and the bound tracer was readily displaced by unlabelled scopolamine. The antigen–antibody reaction was not pH-

*Part 16 in the series "Use of Immunoassay in Plant Science". For Part 15 see R. Atzorn, E. W. Weiler and M. H. Zenk (1981) *Planta Med.* **41**, 1.

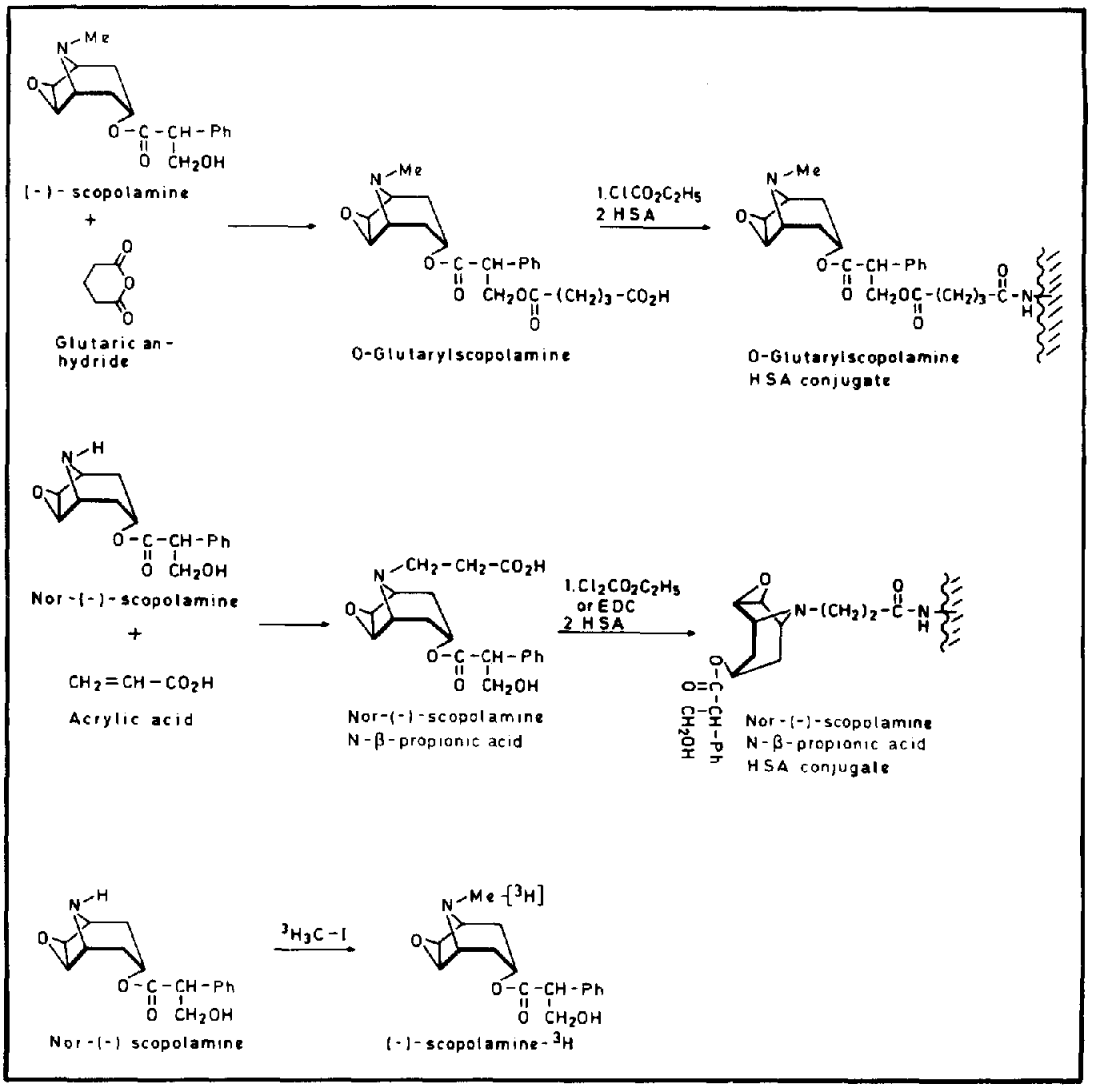


Fig. 1. Synthesis of immunogenic scopolamine–protein conjugates and of tritium labelled [*N*-C^3H_3]scopolamine of high specific activity.

dependent over the range 6.5–8, and optimum results were obtained with phosphate-buffered physiological saline, pH 7.4, as incubation buffer. A selective separation of antibody-bound antigen from free antigen was achieved by precipitation of the immunoglobulin fraction with half-saturated $(NH_4)_2SO_4$, a technique which could be performed at room temperature and which was the method of choice for large assays because it was found to be independent of time. Under the conditions employed, unspecific binding was 0.8–1.0%. In order to have maximum assay specificity, it is essential to incubate assays until the antigen–antibody reaction is in equilibrium. This was the case after incubation for 60 min at room temperature, as judged from the linearity of the standard curve in the logit/log plot (cf. Fig. 2).

Assay sensitivity

A typical standard curve is shown in Fig. 2 using two possible plots. The measuring range of the assay extends from 0.5 to 50 ng of scopolamine (determined as the hydrobromide), and the assay's detection limit at the 99.5% confidence limit is 0.2 ng (0.46 pmol) of scopolamine. Recently, radiolabelled 'scopolamine' of high sp. act. (~50 Ci/mmol) has become commercially available (NEN). However, this material, which actually represents the tritiated methyl chloride of scopolamine, has only a low affinity for the scopolamine-specific antibodies. The commercial material used in the present study (sp. act. 53.5 Ci/mmol) exhibited a *ca* 70-fold higher sp. act. than the [*N*-C^3H_3]scopolamine whose synthesis is described here. However, serum titers are higher only by a factor of 8 and assay sensitivity is increased only by a factor of 5 when the commercial tracer is used, clearly indicating its lower affinity for the antiserum. Moreover, whereas [*N*-C^3H_3]scopolamine is specifically displaced from antibody by scopolamine and shows very little cross-reaction with related tropane alkaloids (see Table 1), the commercial scopolamine methylchloride is displaced to the same extent by 6-hydroxyhyoscyamine and by hyoscyamine, as well as by other tropane alkaloids; i.e. the assay using this material becomes very unspecific. Thus, scopolamine methylchloride is not suitable as a tracer in

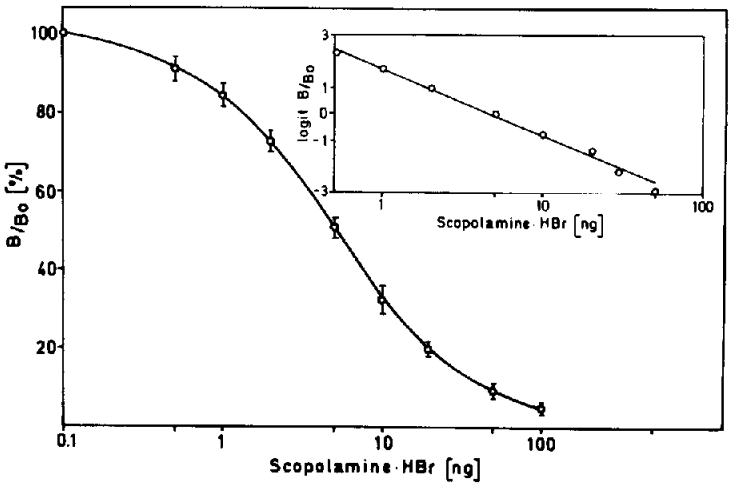


Fig. 2. Standard curve for scopolamine radioimmunoassay.

scopolamine RIA. This effect is explained by the binding of scopolamine methylchloride to antibody populations of low specificity towards scopolamine from which this tracer is readily displaced by a number of structurally similar compounds. It apparently does not bind to the antibodies highly specific for scopolamine present in the serum.

Assay specificity

Since the assay was designed for phytochemical applications, checks of assay specificity were carried out using most of the known related alkaloids available for cross-reactivity studies (Table 1). Table 1 demonstrates two main features of this antiserum. Firstly, since both scopine and tropic acid do not cross-react, the correct stereochemical linkage of both components to form (−)-scopolamine is of utmost importance for immunoreactivity. Secondly, the 6,7-oxy-substituent in the scopine moiety forms the other dominant immunoreactive site of this molecule. Thus, while the biosynthetic intermediate 6-hydroxyhyoscyamine, which occurs only in traces in plant material, still shows some cross-reaction, hyoscyamine, which lacks the 6,7-epoxide, shows more than a 100-fold lower reactivity. While the data in Table 1 give the cross-reactivity of the antiserum fraction used in the present study, it has since been possible to select still more specific antisera which show cross-reactivities of only 1.5% against 6-hydroxyhyoscyamine and of 0.16% against hyoscyamine. All other compounds tested, except for nor-scopolamine, did not cross-react at all. Norscopolamine reacted even better than scopolamine itself. This can be explained because the nitrogen position was used for coupling the scopolamine to the protein to form the immunogen and thus this part of the molecule remained shielded in the conjugate. It is a common observation that compounds differing only in the site chosen for coupling the hapten usually cross-react strongly with the corresponding antibodies [17]. Norscopolamine, which is reported to occur in plants [18], however, has not been detected in any of almost 1000 leaf samples of different plant origin assayed so far for scopolamine by both RIA and an established TLC procedure. Thus, the nor-compound is not likely to cause interference in the assay. Furthermore, plants exhibiting extremely high scopolamine values can easily be checked by TLC for the presence or absence of nor-scopolamine.

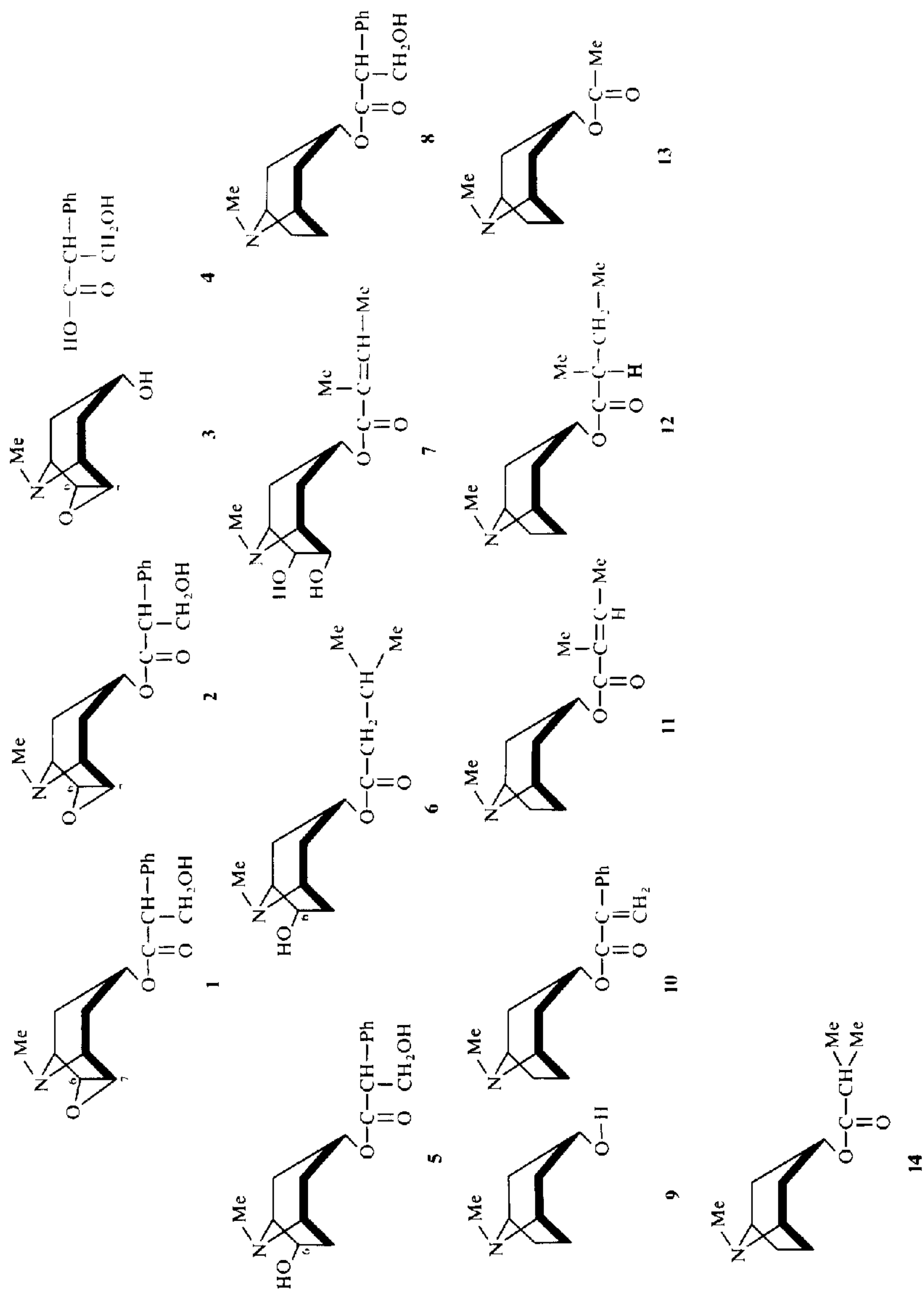
Table 1. Cross-reactions of anti-scopolamine antiserum on a molar basis

Compound	Cross-reaction (%)
Scopolamine (1)	100
Nor-scopolamine (2)	160
Scopine (3)	0
Tropic acid (4)	0
6-Hydroxyhyoscyamine (5)	10.4
Valeroidine (6)	0
Meteloidine (7)	0
Hyoscyamine (8)	0.8
Tropine (9)	0
Apoatropine (10)	0
Tigloidine (11)	0
Valtropine (12)	0
Acetyltropine (13)	0
Butropine (14)	0

In addition to the cross-reactivity studies, unspecific interference in crude plant extracts could be eliminated by assaying the distribution of immunoreactive material on thin layer chromatograms of crude methanolic plant extracts. This experiment has been repeated several times with different samples and so far, the only immunoreactive fraction detected on the chromatograms co-chromatographed with reference scopolamine.

Assay accuracy

Throughout the measuring range, the variability of standard or sample triplicate B/B_0 values averages 2.5% (coefficient of variation) indicating the high precision of the assay. To prove the reliability of the RIA, 101 leaf samples of *Datura sanguinea* and *Duboisia* hybrids were analysed by immunoassay and independently tested in another laboratory using an industrial quality control procedure based on colorimetric determination after TLC separation of scopolamine. RIA determinations were


1
2
3
4
5
6
7
8
9
10
11
12
13
14

performed directly in crude, dilute methanolic extracts. As Fig. 3 demonstrates, both methods correlate closely over the whole range of concentrations found in the samples ($r = 0.955$); the slope of the regression curve ($m = 1.08$) indicates that not only the relative, but also the absolute correlation found was very close. As compared to the TLC procedure, RIA is performed with minimum procedural effort and several 100 quantitative analyses may be obtained per day.

Distribution of scopolamine in plants of Datura *species*

A typical, 7-month-old *Datura innoxia* plant was separated into leaves, stems, roots and flowers. Each single leaf, flower, etc. was extracted freshly and analysed separately for scopolamine. The average values derived from this experiment are shown in Table 2. Leaves and flowers contained highest concentrations of this alkaloid, but it occurred only in small amounts in stems and roots. Over 80% of the total scopolamine of the plant was extracted from the leaves. Drastic differences in alkaloid content of leaves of different age were observed. In young, expanding leaves, located at the shoot tips, as much as 20-fold higher concentrations of scopolamine were found when compared to mature leaves. The young leaves contained, on a per leaf basis higher absolute amounts of the alkaloid than fully expanded ones. This clearly indicates that either export or metabolic breakdown of the alkaloid occurs during leaf development (cf. Fig. 4). Whereas in young leaves scopolamine is mainly found in intercostal areas of the proximal part of the leaf blade, in the mature leaf, the alkaloid is concentrated around or in the leaf veins. This distribution pattern, found in *D. innoxia*, has also been found in *Datura sanguinea*.

These observations extend others made earlier [10, 11]. A decline in leaf scopolamine content with a concomitant increase in hyoscyamine has been reported for ageing plants of *D. innoxia* [6]. Demethylation of scopolamine, also reported to occur in ageing tissues [4], cannot be responsible for our observations since nor-scopolamine would also be immunoreactive.

Within the fruit of *D. innoxia*, highest concentrations of scopolamine are found in the seeds, which thus contain more than two-thirds of the total scopolamine found in the fruit. The concentration of scopolamine markedly decreased (*ca* 20-fold) from the seeds towards the fruit stalk (Fig. 5) and the observation that leaves located near developing fruits usually contain lowest amounts of scopolamine might indicate transport of this alkaloid from leaves to developing seeds.

As a practical consequence of our observations, it should be possible to obtain significantly higher scopolamine yields when young shoots are harvested instead of old ones. Close-growing and mechanical harvesting of the crop would yield higher proportions of young leaves and a better leaf to stem ratio, and would probably allow more than one harvest per year. *Datura sanguinea* grown in fields as bushes or trees would definitely give higher yields when frequently cut.

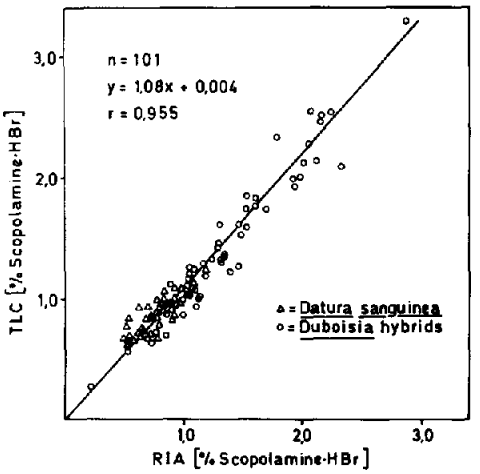


Fig. 3. Correlation between radioimmunoassay and quantitative TLC determination of scopolamine.

Table 2. Distribution of scopolamine within a *Datura innoxia* plant

Part of plant	% of total fr. wt	% of total scopolamine	scopolamine (mg/g fr. wt)
Leaves	43.6	82.6	0.34
Flowers and flower-buds	4.9	9.7	0.89
Stems	37.9	7.8	0.09
Aerial parts, Total	86.4	99.2	0.51
Roots	13.6	0.8	0.02
Whole plant	100	100	0.45

Total fr. wt per plant: 157 g (corresponding to 31 g dry wt); total scopolamine (as HBr): 70 mg; age of plant: 7 months; height of plant: 47 cm.

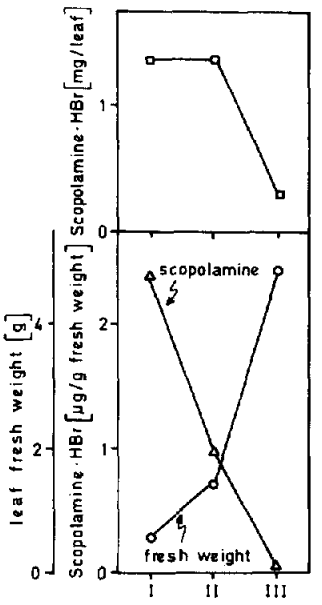


Fig. 4. Concentration and absolute amounts of scopolamine in leaves of increasing age of a *Datura sanguinea* plant (age: 6 months). I → II → III = stages of leaf development.

Part of fruit	Scopolamine (as HBr) (mg/g fresh weight)	(% of total)
stem and fruit bottom	0.054	1.3
outer (green) parts of fruit wall	0.096	4.9
inner (white) parts of fruit wall	0.234	13.3
placenta	0.698	10.6
unripe seeds	1.423	69.9
total fruit:	0.51 mg/g fresh weight	11.4 mg Scopolamine

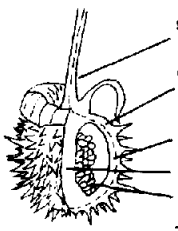


Fig. 5. Distribution of scopolamine within a fruit of *Datura innoxia*.

Diurnal variation of scopolamine in leaves of Datura sanguinea

Phytotron-grown plants of *Datura sanguinea* showed no detectable diurnal variation in scopolamine concentrations either under short-day or long-day conditions (Fig. 6). In this experiment, 20 leaf discs of 5 mm diameter were punched from 20 different leaves per plant and were extracted fresh immediately after harvest. The same set of leaves was used for each measuring point and leaf discs were punched from closely adjacent leaf areas. This technique afforded a means to analyse repeatedly the same set of leaves and thus diminish the extent of leaf-to-leaf variation which would have presented a serious problem had whole leaves been used for analysis. The results clearly show that diurnal changes in leaf scopolamine concentrations do not occur in *D. sanguinea*. Furthermore, the large discrepancies in average scopolamine values found for cuttings of the same mother plant, grown under short-day or long-day conditions (Fig. 6), most likely reflect concentration differences in the batches of the leaves selected for the experiment. Earlier reports on diurnal alkaloid variations [19] might have been influenced by batch-to-batch variations due to harvesting different leaves at different times.

Individual variation of scopolamine content in Datura sanguinea

6-Month-old plants of *Datura sanguinea*, grown in the field in Bochum from seeds collected locally in Ecuador, were analysed for their leaf-scopolamine concentrations. Five leaves per plant were harvested, dried and powdered. Samples of 1 g were extracted and the dilute extracts immunoassayed without any previous purification. A total of 528 plants, harvested within a 2 hr period on the same day, were analysed (Fig. 7). The observed variability in scopolamine content was very substantial. Whereas most plants contained 0.8–1.2% of this alkaloid (the range also normally found in plants growing in their natural climate), a few plants contained lower than 0.2% and others well over 3% of scopolamine.

In the light of the great intra-plant variation of scopolamine concentrations, the whole plant should ideally be analysed to obtain a valid mean value for its scopolamine content. However, this is not feasible in breeding programs. Thus, although the frequency distribution obtained (Fig. 7) might still reflect to some extent an additional variability due to the limited sample size, the heterogeneity of the populations raised from seeds collected from unselected plants is quite obvious. This shows that selection for high-producing individuals from this starting material is very promising. It is remarkable that under the conditions of this experiment plants raised from seeds collected in Ecuador still produced amounts of scopolamine comparable to that of plants grown in their natural environment. This has also been observed for Australian varieties of *Duboisia* species (unpublished results).

EXPERIMENTAL

Mps are uncorr. Immunoassay equipment consisted of semi-automatic pipetting stations, mixers and centrifuges (Analmatic

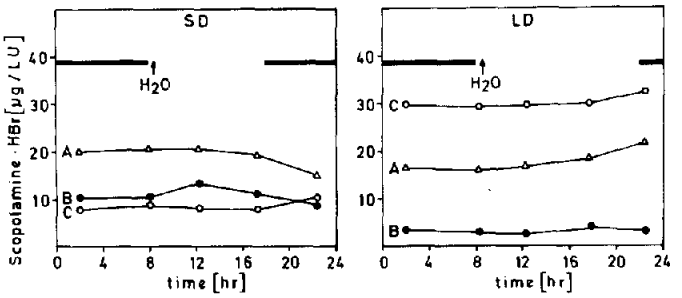


Fig. 6. Diurnal variations of scopolamine in cuttings from mother plants of different origin (A, B, C) grown either under short-day (SD) or long-day (LD) conditions. For each harvest, one leaf disc of 5 mm diameter from 20 previously selected leaves per plant was punched and extracted immediately. The same sets of leaves were used throughout the experiment.

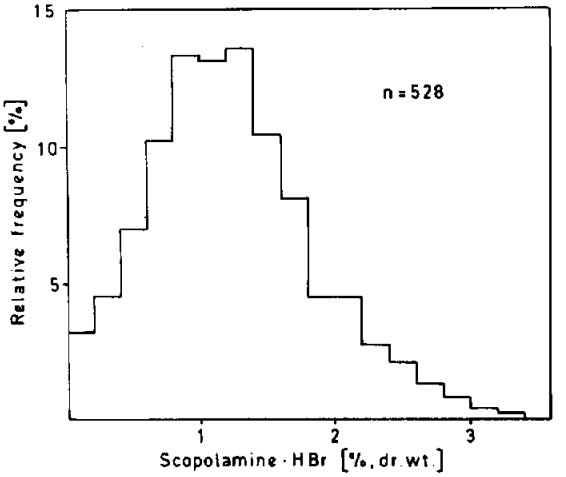


Fig. 7. Scopolamine concentrations in a population of 6-month-old *Datura sanguinea* plants grown from seeds collected from various locations in Ecuador.

system). Radioactivity was determined in a liquid scintillation spectrometer with punched tape output. RIA calculations were done on a programmed off-line calculator using the spline approximation method. TLC was performed on Si G UV_{254} plates (Macherey and Nagel).

Plant material. Seeds of *Datura sanguinea* were collected from mature plants grown naturally in Ecuador. They were sown in the greenhouse and young plants were transferred into the field when 3 months old. *Datura innoxia* Mill., *Duboisia myoporoides* R. Br. and *Duboisia* hybrids were grown in standard soil in the greenhouse at 23° and 65–75% rel. humidity, with no supplementary light.

Extraction procedures. Leaves were dried at 60°, powdered and extracted with 50 ml 80% MeOH per 0.5 g of dry matter, for 30 min under reflux. After filtration, extracts were reconstituted to 50 ml, and aliquots diluted (between 1000- and 10000-fold). Smaller samples up to 100 mg in wt (leaf discs, parts of plant organs) were extracted fresh in 5 ml 80% MeOH at 60° for 15 hr. After reconstitution and dilution, aliquots were immunoassayed. For a few expts, fresh samples of fruits or other parts of the plant were extracted 2 × for 30 min under reflux with suitable vols of 80% MeOH, and the filtered and combined extracts were processed further as above.

Chemicals and immunochemicals. Scopolamine HBr used for synthetic work and as immunoassay standard was supplied by Boehringer, [^{3}H]MeI (sp. act. 1.13 Ci/mmol) was purchased from New England Nuclear. HSA (100% pure) was obtained from Behringwerke, Marburg, and *N'*-ethyl-*N*(3-dimethyl)amino-propyl-carbodiimide hydrochloride from Merck, Darmstadt. Complete Freund's adjuvant was obtained from Difco and bovine serum from Mediapharm. Minisolve (Koch-Light) was used as scintillator. All other chemicals and reagents were of highest purity available.

Synthesis of [N-C^3H_3]-(−)-*scopolamine.* Nor-(−)-scopolamine was prepared from scopolamine according to ref. [20] and crystallized from EtOAc, mp 110–112° (lit. [20] 112–114°). IR cm^{-1}: 1720, 1175, 1048, 868, 848, 735, 697; MS *m/z* (rel. int.): 289 (M^+, 7), 124 (54), 123 (85), 122 (100), 118 (13), 106 (26), 103 (22), 94 (55), 80 (79). Nor-(−)-scopolamine (51 mg, 176.5 μmol), dissolved in 1 ml Et_2O–$CHCl_3$ (4:1), was treated for 24 hr at 25° and 68 hr at 40° in a sealed all-glass apparatus with [^{3}H]MeI (12.57 mg, 88.5 μmol, sp. act. 1.13 Ci/mmol) using a modification of the procedure of ref. [21]. The reaction mixture was dried under N_2, redissolved in MeOH and purified by TLC in the solvent system cyclohexane–$CHCl_3$–diethylamine, 5:4:1 (R_f = 0.51). The isolated [*N*-C^3H_3]-(−)-scopolamine was over 99% pure (yield: 71%, sp. act. 0.67 Ci/mmol).

Coupling of nor-(−)-scopolamine to HSA. Synthesis of nor-(−)-scopolamine-N-β-propionic acid. Nor-(−)-scopolamine (2 g, 6.92 mmol) was dissolved in 4 ml EtOH and a soln of 577.6 mg (8.3 mmol) acrylic acid, dissolved in 1 ml EtOH was added. After reaction for 5.5 hr at 70°, another 385 mg (5.54 mmol) acrylic acid in 0.65 ml EtOH was added and the mixture reacted for 2 hr at 70°, 43 hr at 20° and 20 hr at 4°. The crystallized nor-(−)-scopolamine-*N*-β-propionic acid was isolated (1.49 g, 60% yield), mp 174°. IR cm^{-1}: 1725, 1630, 1367, 1270, 1200, 1067, 1010, 855, 700; MS *m/z* (rel. int.): 361 (M^+, 4), 302 (5), 289 (4), 284 (2), 272 (2), 212 (10), 196 (20), 166 (16), 154 (22), 152 (20), 124 (30), 123 (42), 122 (66), 106 (28), 103 (32), 94 (52), 91 (34), 80 (100). $[\alpha]_{589}$ −25.2° (*c* 3.2 in H_2O). Found: C, 63.15; H, 6.55; N, 3.90; O, 26.57. Calc. for $C_{19}H_{23}N_6$: C, 63.10; H, 6.41; N, 3.88; O, 26.62%. Methylation with CH_2N_2–Et_2O gave the Me ester: MS *m/z* (rel. int.): 375 (M^+, 16), 302 (26), 284 (20), 226 (35), 210 (61), 180 (40), 166 (49), 154 (100), 136 (25), 121 (61), 118 (46), 106 (54), 103 (96), 94 (44).

Coupling procedure A. Nor-(−)-scopolamine-*N*-β-propionic acid (64 mg, 177.5 μmol) was dissolved in 1.15 ml DMF and mixed with 18.04 mg (177.5 μmol) triethylamine. The soln was cooled (0°) and 20.1 mg (184.5 μmol) *iso*-butylchlorocarbonate were added with stirring. After 20 min, the soln containing the mixed anhydride was added dropwise to 88.5 mg (1.28 μmol) HSA dissolved in 40% aq. pyridine (0°). During the addition, the pH was kept at 7.5 by adding 1.9 ml $NaHCO_3$ (1 mol/l). After stirring at 4° for 24 hr the reaction mixture was dialysed for 5 days against H_2O. In a second synthesis, the formation of the mixed anhydride was stopped after 10 min, and the pH during the coupling to the protein was kept between 7 and 7.5 with NaOH (1 mol/l.).

Coupling procedure B. To a soln of 88.7 mg (1.28 μmol) HSA in 3.55 ml H_2O adjusted to pH 10.7 with NaOH (1 mol/l.) was added 64 mg (177.5 μmol) nor-(−)-scopolamine-*N*-β-propionic acid in 0.95 ml H_2O. To the soln (pH 7.5) was added 75 mg *N*-ethyl-*N'*-(3-dimethyl)aminopropyl-carbodiimide hydrochloride in 4 portions. After stirring at 4° for 18 hr, the mixture was dialysed against H_2O for 4 days. The 3 conjugates were pooled and further diluted with Pi-buffered saline, pH 7.4 (protein concn *ca* 2 mg/ml) for immunization.

Synthesis of glutaryl-(−)-scopolamine. (−)-Scopolamine (303 mg, 1 mmol) and glutaric acid anhydride (228 mg, 2 mmol) were dissolved in 1.5 ml C_6H_6 and allowed to stand 24 hr at room temp. After addition of 0.5 ml MeOH, the mixture was chromatographed (EtCOMe–MeOH–NH_3 (7.5%), 6:3:1) and the product (R_f = 0.4) eluted with MeOH to yield 210 mg (yield 50%) of a yellow oil. MS *m/z* (rel. int.): 417 (M^+, 4), 285 (36), 154 (36), 138 (55), 108 (45), 103 (54), 94 (100).

Coupling of glutaryl-(−)-scopolamine to HSA. Glutaryl-(−)-scopolamine (208.5 mg, 0.5 mmol), dissolved in 3 ml DMF and 50.75 mg (0.5 mmol) triethylamine were cooled (0°) and 56.5 mg (0.52 mmol) *iso*-butylchlorocarbonate was added. After 10 min, the soln was added dropwise to an ice-cold soln of 250 mg (3.62 μmol) HSA, dissolved in 10 ml 40% aq. pyridine. The pH was kept constant at 7.8 by the addition of 7.5 ml $NaHCO_3$ (1 mol/l.). After reaction for 24 hr at 4°, the mixture was dialysed 21 hr against 50 mM Pi (pH 8) and finally 4 days against H_2O. For immunization, the conjugate soln was diluted with Pi-buffered saline to a protein concn of *ca* 2 mg/ml.

Immunization and antiserum production. The conjugate prepns were administered to 12 to 16-week-old rabbits as a 1:1 emulsion in Freund's complete adjuvant. After 4 weekly

intradermal immunizations, intramuscular booster injections were given monthly and blood was collected 1 and 2 weeks after each booster. The collected serum was stored at −18° and was found to be stable under the conditions employed.

Performance of RIA. Details of the procedure have been described earlier [12, 13]. In brief, the assay was carried out as follows: incubation tubes received 0.5 ml Pi-buffered saline, pH 7.4 (PBS, 0.01 M phosphate, 0.15 M NaCl), 0.1 ml dilute bovine serum, 0.1 ml dilute [*N*-C^3H_3]scopolamine and 0.1 ml standard or sample. After mixing, 0.1 ml dilute antiserum was added, the tubes were mixed again and incubated for 1 hr at room temp. followed by the addition of 1 ml $(NH_4)_2SO_4$ soln (10 vol. satd soln plus 1 vol. H_2O to prevent crystallization during pipetting). After mixing, tubes were incubated for 30 min at room temp. and then centrifuged. The pellets were washed once with 1 ml half-satd $(NH_4)_2SO_4$, dissolved in 0.2 ml of H_2O and mixed with 1 ml of scintillation fluid. After thorough mixing, the tubes were counted for radioactivity. Under the conditions employed, counting efficiency was 14%.

Acknowledgements—We are most grateful to Dr. W. J. Griffin, Brisbane, Australia and to Prof. W. C. Evans, Nottingham, England, for providing the rare tropane alkaloids. The financial support by the Bundesminister für Forschung und Technologie, Bonn, is greatly appreciated. Our thanks are also due to Mr. Pablo S. Jourdan, Tampa, Florida, for his help in preparing the English version of the manuscript and C. H. Boehringer Sohn Corp., Ingelheim, for the quantitative TLC determinations of scopolamine.

REFERENCES

1. Griffin, W. J. (1976) *Econ. Botany* **30**, 361.
2. Mothes, K. and Schütte, H. R. (1969) *Biosynthese der Alkaloide*. Verlag der Wissenschaften, Berlin.
3. Romeike, A. (1960) *Planta Med.* **8**, 491.
4. Verzár-Petri, G. (1973) *Pharmazie* **28**, 603.
5. Gupta, S., Prabhakar, V. S. and Madan, C. L. (1973) *Planta Med.* **23**, 370.
6. Cucu, V. and Paun, E. (1968) *Planta Med.* **16**, 338.
7. Puech, A., Jacob, M. and Gandy, D. (1972) *J. Chromatogr.* **68**, 161.
8. Allport, N. L. and Wilson, E. S. (1939) *J. Pharm. Pharmacol.* **12**, 399.
9. Griffin, W. J., Brand, H. P. and Darl, J. G. (1975) *J. Pharm. Sci* **64**, 1821.
10. Karawya, M. S., Abdel-Wahab, S. M., Hifnawy, M. S. and Ghourab, M. G. (1975) *J. Assoc. Off. Analyt. Chem.* **58**, 884.
11. Padula, L. Z., Bandoni, A. L., Rondina, R. V. D. and Coussio, J. D. (1976) *Planta Med.* **29**, 357.
12. Weiler, E. W. and Zenk, M. H. (1976) *Phytochemistry* **15**, 1537.
13. Arens, H., Stöckigt, J., Weiler, E. W. and Zenk, M. H. (1978) *Planta Med.* **34**, 37.
14. Westekemper, P., Wieczorek, U., Gueritte, F., Langlois, N., Langlois, Y., Potier, P. and Zenk, M. H. (1980) *Planta Med.* **39**, 24.
15. Weiler, E. W. (1980) *Planta* 148, 262.
16. Weiler, E. W. and Mansell, R. L. (1980) *J. Agric. Food Chem.* **28**, 543.
17. Kahn, D., Andrieu, J. M. and Dray, F. (1974) *Immunochemistry* **11**, 327.
18. Gmelin, R. (1940) *Arch. Pharm.* **279**, 112.
19. Birecka, H. and Zebrowska, Z. (1960) *Bull. Acad. Polon. Sci. Ser. Sci. Biol.* **8**, 339.
20. Banholzer, R., Schulz, W. and Zeile, K. (1969) *German Offen.* 1, 912, 563 (Cl. C 07d).
21. Werner, G. and Mohammad, N. (1966) *Liebigs Ann. Chem.* **694**, 157.