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.

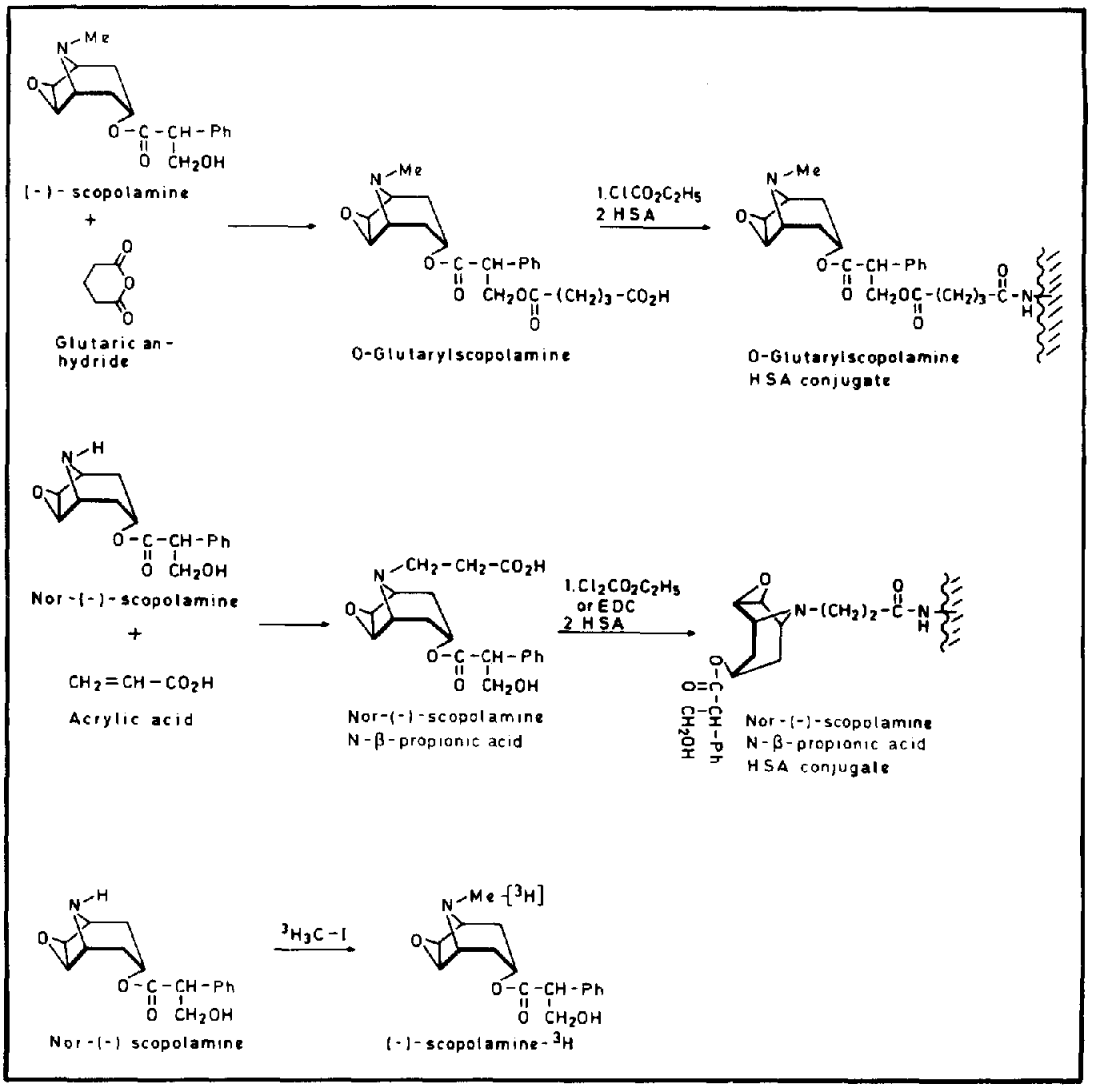

Fig. 1. Synthesis of immunogenic scopolamine–protein conjugates and of tritium labelled [*N*-C^3H_3]scopolamine of high specific activity.

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