Number of male *Callisaurus draconoides* possessing recrudesced or regressing testes after receiving daily injections of melatonin or saline for 20 days

Treatment	Testicular state	
	Recrudesced	Regressing
Saline	7	4
Melatonin	2	8

at each injection. Food (*Tenebrio* larvae) was provided at regular intervals, and only data for healthy animals are reported here. At the end of 20 days, the lizards were killed by etherization, and their testes were removed, weighed individually, and fixed in formalin. Since there was no difference between experimental and control animals in combined testicular weights, reproductive condition of the lizards was assessed by histologic examination of the gonads. The left testis from each animal was embedded in paraffin and sectioned at 7 μm. Median cross sections were mounted on glass slides and stained with Mayer's hematoxylin and eosin-Y. Prior to examination of the slides, the label on each was masked so that all examinations were made without knowledge of the group to which a particular donor animal belonged.

Initial examination of the sectioned material revealed that some lizards possessed maximally recrudesced testes at the time of sacrifice, whereas others were undergoing gonadal regression. In maximally recrudesced testes, the seminiferous lumen was relatively packed with sperm, the seminiferous epithelium was well-developed, and all cell types (insofar as they can be distinguished) were abundant. Additionally, some spermatigonial mitoses were evident, and the interstitium was well-endowed with Leydig cells. Conversely, testes in regression contained fewer sperm, the seminiferous epithelium was reduced (with a concomitant reduction in the number of cells and of cell-types present), and many fewer Leydig cells were evident.

The testes were assigned to different categories on the basis of these apparent differences in histologic condition (table), and the proportional representation of experimental and control animals in these categories was assessed using Fisher's exact test[13]. This statistical procedure indicates that significantly ($p = 0.05$) more lizards injected with melatonin exhibited testicular regression than in the control group.

The samples used in this preliminary experiment were rather small, and so the results must be viewed with caution. Nevertheless, the low probability of obtaining the observed frequency distribution (table) by chance alone indicates that melatonin may exert an antigonadotrophic effect in male *Callisaurus draconoides* similar to that reported for other ectothermic vertebrates[8–10].

13 R. R. Sokal and F. J. Rohlf, Biometry. W. H. Freeman & Co., San Francisco 1969.

PRO EXPERIMENTIS

Analysis of abscisic acid and indole-3-acetic acid from fruits of *Vitis vinifera* L. by high pressure liquid chromatography

H. Düring

Federal Research Institute of Grape Breeding, D–6741 Siebeldingen (Federal Republic of Germany), 9 March 1977

Summary. Simultaneous quantitative analysis of the plant hormones abscisic acid and indole-3-acetic acid from fruits of *Vitis vinifera* ssp. was performed by thin layer chromatography and high pressure liquid chromatography. Hormonal activity was proved in a biological test.

Biogenous plant hormones are suggested to play an important role in developmental and ripening processes of fleshy fruits[1,2]. In grape berries, auxin-, gibberellin- and cytokinin-like substances have been detected mainly by their activities in biological tests[3–6], while abscisic acid (ABA) and ethylene were determined by gas-liquid-chromatography[7]. In recent years, high pressure liquid chromatography (HPLC) has been introduced in plant hormone analysis, especially for cytokinins[8–10] and abscisic acid[11]. In this study, a method is described by which 2 hormones, ABA and indole-3-acetic acid (IAA), were simultaneously extracted, identified and quantitatively determined by HPLC. In addition the growth regulating activity of the extracted IAA was confirmed by a biological test.

Material and methods. Berries of *Vitis vinifera* L., cv. Bacchus, were harvested from fieldgrown grapevines of the institute. Berries were cleaned by dest. H_2O and kept frozen at $-20\,°C$ until extraction. Frozen berries (20 g fresh weight) were homogenized in 60 ml dest. H_2O and extracted in hot water (98–100 °C) for 20 min. After filtration under vacuum and a reextraction of the residues, the acidified filtrate (pH 3.0 by 2 N H_2SO_4) was continuously extracted by ether for 20 h in a liquid-liquid extractor. The ether fraction was then extracted by a saturated $NaHCO_3$-solution (5 times) and dest. H_2O alternately (5 times). The acidified water fraction (pH 3.0 by 2 N H_2SO_4) was again continuously extracted by ether for 20 h. The ether fraction was evaporated and subjected to thin layer chromatography (TLC) (Kieselgel HF 254, 200 μm, Merck) using n-propanol : NH_3(25%) : H_2O 80:10:10 v/v according to Wakhloo[12]. The extracts, as well as synthetic IAA and ABA, were spotted on TLC-plates as 2–3 cm bands and developed in chambers under saturated atmospheric conditions. The zones seen under UV 254 were marked and partly identified by their R_f-values and by spraying Ehrlich reagent (1% p-dimethylaminobenzaldehyde in conc. HCl + 96% ethanol mixed 1:1). Spots from unsprayed plates were scraped off, eluted by ether and after evaporation diluted in 0.1 ml methanol for HPLC-analysis. Details are given in figure 1. Peaks were identified by comparing retention times of synthetic and natural compounds. In addition peak identity was confirmed by collection of HPLC-separated IAA using a fraction collect valve and subjection to the Avena straight growth test according to Larsen[13].

Results and discussion. Since methanol as an organic solvent is reported to produce possibly methyl abscisate[14,15], and to destroy free IAA[16], a hot water extraction was chosen which does neither destroy ABA nor IAA (Coombe, personal communication and own results); moreover extraction time is shortened. Continuous extraction of the acidified water fraction by ether was preferred to funnel shaking because of higher yields (up to 15%) of both, ABA and IAA. Separation and first identification of ABA and IAA is possible by means of TLC with cochromatographed authentic compounds. After spraying Ehrlich reagent, the IAA containing zone at R_f 0.40–0.50 turned red-blue while the ABA containing zone can easily be found at R_f 0.55–0.60 under UV 254. In a few samples only, taken during the ripening period in September, traces of indole compounds apart from R_f-values of IAA occurred on TL-plates. The R_f-values of these compounds located at the front of the solvent system and the colour reaction (red zones after spraying Ehrlich reagent) preliminary indicate the presence of 3-indolylacetonitrile and/or ethyl-3-indolylacetate[3,17]. HPLC-analysis of the ABA- and IAA-containing zones (R_f 0.40–0.60) shows that both hormones can be separated using anionexchange chromatography (figure 1). Peak height was chosen for quantitative estimations using the calibration curves given in figure 2. The minimum detectability of ABA was found to be 1 ng and 25–50 ng of IAA, respectively. While the sensitivity of the single-wavelength (254 nm) UV-photometer exhibited a high sensitivity for ABA, a multi-wavelength detector providing 280 nm is assumed to improve minimum detectability of IAA.

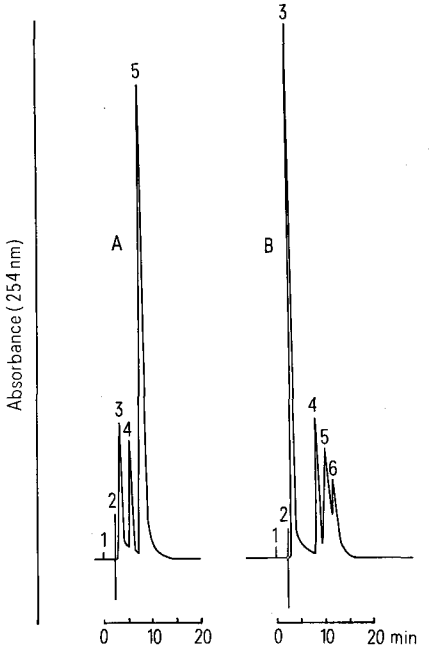

Fig. 1. Separation of extracted (A) and authentic (B) abscisic acid (ABA) and indole-3-acetic acid (IAA). A *1* Start, *2* front, *3* ABA, *4* unknown, *5* IAA. – B *1* Start, *2* front, *3* ABA, *4* IAA, *5* IPrA (indole-3-propione acid), *6* IBA (indole-3-butyric acid). Operating conditions: Instrument: Du Pont 841 Liquid Chromatograph. Column: Permaphase AAX anion-exchange packing (1 m × 2.1 mm I.D., Du Pont). Mobile phase: NaH_2PO_4 (0.9 g/l H_2O). Detector: UV-photometer at 254 nm. Sensitivity: 0.08 AUFS. Chart speed: 100 mm/h. Column pressure: 1000 psi (70.4 atm). Flow rate: 0.56 ml/min.

Date	ABA (µg/100 g fresh weight)	IAA (µg/100 g fresh weight)
30 July	11.3	16.75
27 August	27.7	9.00
27 September	10.5	3.50

As shown in the table, the contents of ABA and IAA change during berry development. While IAA is reported to increase in the first period of berry growth and to decrease before the start of ripening[3,18], ABA accumulation in grape berries was observed (in parallel to sugar accumulation) in the period of ripening[7,19]. The datas given in the table confirm these results.

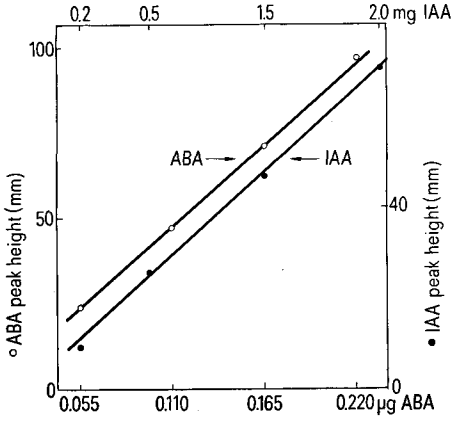

Fig. 2. Calibration curves of abcisic acid (ABA) and indole-3-acetic acid (IAA). Injection volumes: 2–15 µl. For operating conditions see figure 1.

1 B. G. Coombe, A. Rev. Pl. Physiol. *27*, 507 (1976).
2 J. A. Sacher, A. Rev. Pl. Physiol. *24*, 197 (1973).
3 J. P. Nitsch, C. Pratt, C. Nitsch and N. J. Shaulis, Am. J. Bot. *47*, 566 (1960).
4 B. G. Coombe, Pl. Physiol. *35*, 241 (1960).
5 S. Iwahori, R. J. Weaver and R. M. Pool, Pl. Physiol. *43*, 333 (1968).
6 G. Waitz, Doctoral thesis, University of Hohenheim (1975).
7 B. G. Coombe and C. R. Hale, Pl. Physiol. *51*, 629 (1973).
8 M. G. Carnes, M. L. Brenner and C. R. Andersen, J. Chromat. *108*, 95 (1975).
9 J. S. Challice, Planta *122*, 203 (1975).
10 H. Hahn, Pl. Cell Physiol. *17*, 947 (1976).
11 H. Düring and O. Bachmann, Physiol. pl. *34*, 201 (1975).
12 J. L. Wakhloo, Planta *65*, 301 (1965).
13 P. Larsen Encycl. Pl. Physiol. *14*, 521 (1961).
14 K. Dörfling, Prog. Bot. *38*, 148 (1976).
15 B. V. Milborrow and R. Mallaby, J. exp. Bot. *26*, 741 (1974).
16 H. Zimmermann, Chr. Siegert and R. Karl, Z. Pfl. Physiol. *80*, 225 (1976).
17 J. A. Bentley, Encycl. Pl. Physiol. *14*, 501 (1961).
18 G. Alleweldt and H. A. A. Hifny, Vitis *11*, 10 (1972).
19 H. Düring, Vitis *13*, 112 (1974).