SC (Si gel, Akt. St. II) und anschließend weiter durch DC (Si gel, GF 254) als Laufmittel dienten Et_2O–petrol (= E–P)-Gemische. 150 g Wurzeln ergaben 3.5 g **1** und 3.8 g **2** (E–P 1:1). 300 g oberirdische Teile lieferten 15 mg **3**, 18 mg **1**, 10 mg **2** und 0.5 g **4**.

*3β-[2-Acetoxymethyl-*trans*-crotonyloxy-]-dihydro-euparin* (**2**). Farbloses Öl, *IR*. OH 3500–2600: OAc 1750: $C{=}CCO_2R$ 1720, 1640; $>C{=}O$ 1650 cm^{-1} *MS*. M^+ *m/e* 374.137 (3%) (ber. für $C_{20}H_{22}O_7$ 374.137); $-{}^{\cdot}CH_3$ 359(5); $-MeCH{=}C(CH_2OAc)CO_2H$ 216 (100); 216 $-{}^{\cdot}CH_3$ 201 (91); $MeCO^+$ 43 (95).

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TERPENOID LACTONES AS PLANT GROWTH REGULATORS

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Key Word Index—*Saussurea lappa*; *Phaseolus aureus*; costus root oil; terpenoid lactones; plant growth regulators.

Abstract—Plant growth regulating activity of dehydrocostus lactone possessing an α-methylene-γ-lactone moiety has been compared with its two derived C-16 lactones, in which a trisubstituted double bond and a cyclopropane ring are conjugated with the lactone carbonyl. The results show that the two latter compounds are slightly more active than dehydrocostus lactone.

INTRODUCTION

Compounds having a terpenoid skeleton and a lactone moiety as their structural specificity, are emerging as a group of plant growth regulators [1]. It has been shown that this activity of these terpenoid lactones is due to the exomethylene group conjugated to the lactone carbonyl and this structural feature is almost indispensable for this action [2]. The present investigations were made to learn if conjugated lactones other than the α-methylene containing group were equally effective in regulating growth activities such as rooting of mung bean cuttings. No reports on this type of work have appeared in the literature and it was hoped that this investigation would help to clarify the structural specificity required for growth activity of the terpenoid lactones.

RESULTS AND DISCUSSION

The present work confirms the dependence of plant growth regulating activity of the terpenoid lactones upon the presence of a α-methylene group in conjugation with the lactone carbonyl. This was seen (Fig. 1) in the case of the root forming potential of dehydrocostus lactone (**1**) which fell off as the exocyclic double bound in conjugation with the lactone carbonyl was removed either by forming a pyrazoline derivative (**2**) or by its reduction to give dihydrodehydrocostus lactone (**5**) which had identical activity compared with **2**. It is interesting to note that the C-16 lactones (**3** and **4**) in which the

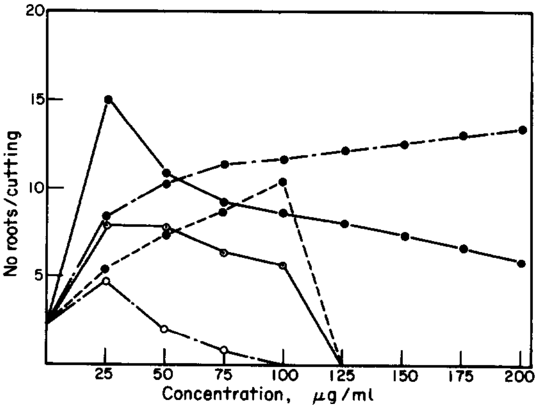

Fig. 1. Effect of terpenoid lactones on rooting in hypocotyl cuttings of *Phaseolus aureus* Roxb ●—● compound 3, ●–·–● compounds 4, ●--● costus root oil, ○—○ dehydro costus lactone, ○–·–○ adduct of dehydro costus lactone with diazomethane.

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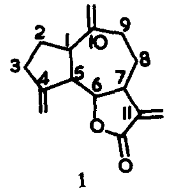

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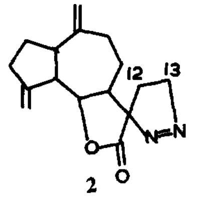

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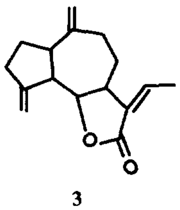

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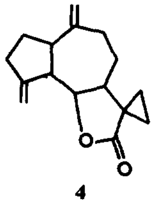

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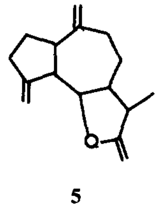

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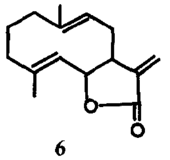

6

conjugation is of different type were also active as plant growth regulators and in fact their activity was slightly more than the parent α-methylene-γ-lactone, dehydrocostus lactone (1).

As a result of large scale screening of various essential oils as plant growth regulators it was found that the oil extracted by low temperature solvent extraction [3] from the costus roots (*Saussurea lappa*) showed considerable activity which could be easily assigned to the presence of dehydrocostus lactone (1) and costunolide (6). The present work was done on dehydrocostus lactone which is the major crystalline lactone of the oil (35%) as compared with costunolide which occurs only to the extent of 15%.

Dehydrocostus lactone on reaction with diazomethane afforded a crystalline pyrazoline derivative (2) in quantitative yield. The IR spectrum showed the expected intense band at 1775 cm^{-1} (γ-lactone) while the strong band of the parent lactone at 810 cm^{-1} due to conjugated α-methylene-γ-lactone was absent. Its NMR spectrum was also in complete accord with this structure.

The pyrazoline derivative on pyrolysis at 100° afforded a two component mixture. Chromatographic separation gave two crystalline products of the molecular formula $C_{16}H_{20}O_2$ (3) and (4). Compound 3 in its IR spectrum showed bands at 1760 (γ-lactone), 1640 and 890 cm^{-1} (methylenic double bond) and its NMR spectrum displayed a doublet of quartets at 5.93 δ (1H, $J = 8$ and 5 Hz) and a doublet of doublets at 1.03 and 1.0 δ (3H, $J = 8$ and 3 Hz) for the proton and the methyl group respectively on the newly created trisubstituted double bond. The γ-lactone (4), in its IR spectrum, interestingly showed a band at a considerably lower frequency (1755 cm^{-1}) probably due to conjugation with the cyclopropane ring. Its NMR spectrum confirmed this structure by displaying a 4H complex between 0.7 to 1.4 δ due to the protons on the cyclopropane ring.

Dihydro-dehydrocostus lactone (5) was prepared according to known procedures and all the compounds were tested as plant growth regulators (Fig. 1).

EXPERIMENTAL

Mp's are uncorrected. IR spectra were run in Nujol on crystalline compounds. NMR spectra were registered on a 60 MHz apparatus using TMS as internal standard. For column chromatography Si gel was used.

Dehydrocostus lactone (1). Dehydrocostus lactone (mp 61°; $[\alpha]_D$ −13°) was isolated from costus root oil as described earlier [3] and was identified by its superimposable IR spectrum on that of the authentic sample and by mmp determination.

Pyrazoline derivative (2). To a soln of dehydrocostus lactone (2 g) in Et_2O (20 ml) was added dropwise a soln of CH_2N_2 in Et_2O till the soln attained a yellow colour. After 0.5 hr the solvent was evaporated to afford a product (2.1 g) which immediately solidified. It was filtered through a bed of Si gel and crystallized from Et_2O–petrol mixture to afford a pure compound (TLC) mp 92°. (Found: C, 71.1; H, 6.96. $C_{16}H_{20}O_2N_2$ requires: C, 70.56; H, 7.40%). IR ν_{max}^{Nujol} 1775, 1640, 890, 880 cm^{-1}. NMR: δ 5.3 and 5.07 (2H, C-4 = CH_2) 4.83 (2H, C-10 = CH_2) and *t* at 4.6 (3H, $J = 8$ Hz C-6 and C-13).

Pyrolysis of the pyrazoline derivative. The pyrazoline derivative (2.3 g) was heated in an oil bath at 100° for 7 hr. The product was cooled and chromatographed over Si gel (100 g). Elution of the column with petrol–Et_2O (95:5) afforded compound 3, mp 74°, in the initial 10 × 25 ml fractions. (Found: C, 79.01; H, 8.31. $C_{16}H_{20}O_2$ requires: C, 78.65; H, 8.25%). IR ν_{max}^{Nujol}: 3080, 1760, 1640, 990, 900 and 890 cm^{-1}. NMR: δ 5.93 (1H *dq* $J = 8$ and 3 Hz, C-12 $>$C=C$<$H) 5.25 and 5.0 (2H, C-4 = CH_2) 4.83 and 4.77 (2H, C-10 = CH_2) 3.8 (1H, *t*, $J = 8.5$ Hz, C-6) and 2.1 (3H, *dd*, $J = 8$ and 3 Hz, allylic methyl). Subsequent elution the column with the same solvent afforded compound 4, mp 70°, in the tail fractions. (Found: C, 79.00; H, 8.49. $C_{16}H_{20}O_2$ requires: C, 78.65; H, 8.25%). IR ν_{max}^{Nujol}: 3075, 1755, 1660, 1630. 990, 900 and 890 cm^{-1}. NMR: δ 5.27 and 4.97 (2H, C-4 = CH_2), 4.8 and 4.73 (2H, C-10 = CH_2) 3.93 (1H, *t*, $J = 9$ Hz, C-6) and at 0.7–1.4 (4H, *m*, cyclopropane protons).

Bioassay of the compounds. Stock solns of the compounds were made by dissolving a weighed quantity of the compound in EtOH and the volume was made up with phosphate buffer soln (pH 6.5). All the dilutions were made with the phosphate buffer. Mungbean (*Phaseolus aureus* Roxb.) hypocotyl-rooting bioassay [4] tests were employed. Mungbean var. Sathi was grown in sterilized silica sand for 7 days on 16 hr photoperiod at 25° and 20° night temp. at 32 × 10 lx. The cotyledons were removed leaving two primary leaves and the hypocotyls were cut 4 cm below the cotyledonary node. Four uniform cuttings were placed in glass vials containing 5 ml of the specific strength of chemical soln. Phosphate buffer (pH 6.5) was used as control. Roots 3 mm or longer were counted after 6 days of incubation. All experiments were conducted in a completely randomized block design with three replications.

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EINE NEUE DITERPENSÄURE AUS *PERYMENIUM ECUADORICUM**

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Key Word Index—*Perymenium ecuadoricum*; Helianthcae; Compositae; diterpenes.

Die große Tribus Heliantheae wird in viele Subtribus aufgeteilt [1]. Seit einiger Zeit beschäftigen wir uns mit der Untersuchung möglichst vieler Gattungen, um festzustellen, wieweit die Art der Inhaltsstoffe mit der Einteilung parallel geht und welche Anhaltspunkte für die teilweise geplante Eingruppierung von Gattungen der früheren Tribus Helenieae sich abzeichnen.

Vertreter der Gattung *Perymenium* (Subtribus Verbesininae) sind bisher noch nicht untersucht worden. Die Wurzeln von *P. ecuadoricum* Blake enthalten das weitverbreitete Pentainen **1**, sowie ein komplexes Gemisch mehrerer Diterpene.

Nach sorgfältiger dünnschichtchromatographischer Trennung erhält man die bekannten Verbindungen **2**–**5**. Die polaren Anteile enthalten jedoch noch zwei weitere Säuren, die zusätzlich einen Angelicaesterrest enthalten. Erst nach Veresterung mit Diazomethan konnten die entsprechenden Methylester in reiner Form isoliert werden. Der weniger polare Ester ist offenbar identisch mit dem Methylester einer aus *Enhydra fluctuans* isolierten Säure [2]. Der polarere Ester enthält einen Sauerstoff mehr. Alle Daten sprechen für das Vorliegen von **9**, so daß dem Naturstoff die Konstitution **8** zukommen muß. Wir möchten die Säure Perymeniumsäure nennen.

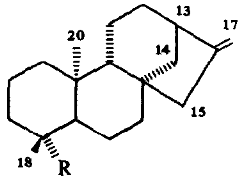
6 haben wir auch aus einer *Silphium*-Art zusammen mit den entsprechenden Isovaleriansäure ester isoliert.

Die oberirdischen Teile enthalten Germacren D [4] sowie **2** und **4**.

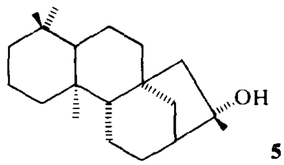
Diterpene vom Typ **2**–**5** haben wir auch aus einigen *Verbesina*-Arten isoliert [3]. Allerdings kommen derartige Verbindungen auch in vielen Vertretern, die zu anderen Subtribus gehören, vor.

Weitere Untersuchungen müssen zeigen, ob die speziellen Verbindungen **6** und **8** chemotaxonomische Bedeutung besitzen.

$Me[C\equiv C]_5CH=CH_2$ **1**



2:R = CHO
3:R = CH$_2$OH
4:R = CO$_2$H



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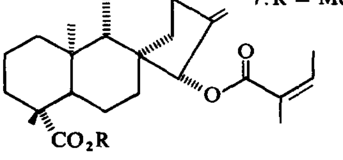
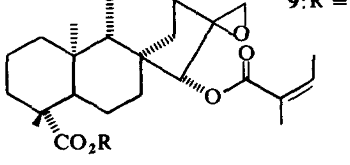


6:R = H
7:R = Me



8:R = H
9:R = Me

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