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CHEMICAL CONSTITUENTS OF THE GENUS *DAHLIA*—III.*

A CHEMOTAXONOMIC EVALUATION OF SOME *DAHLIA COCCINEA* STRAINS

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Abstract—An examination of five strains of *Dahlia coccinea* for polyacetylenes reveals a close chemical similarity between the five morphological types, especially in the tubers. Some differences were found in the aerial parts.

INTRODUCTION

MANY acetylenic compounds have been isolated from Compositae, to which family the genus *Dahlia* Cav. belongs. The main purpose of this investigation is to examine similarities and differences within a morphologically closely related group of plants.

The genus *Dahlia* originated in Mexico, and it has been thoroughly described by Sherff.[1] He describes eighteen species besides some varieties, and divides the genus *Dahlia* Cav. into three sections: Pseudodendron (three species), Epiphytum (one species), and Dahlia (fourteen species). The many horticultural varieties (about 15,000) which are known, belong to the last section. These cultivars seem largely to have been derived from two species: *Dahlia pinnata* Cav. and *D. coccinea* Cav.

D. lehmanni Hieron.[2] and *D. imperialis* Roezl (unpublished), belonging to section Pseudodendron, have been investigated previously but no polyacetylenic compounds were found. The results from the investigation of other *Dahlia* species, including *D. coccinea* L.,† have also been reported.[2–4]

DISCUSSION AND RESULTS

The acetylenic compounds, which have been characterized in the five strains of *Dahlia coccinea* examined are listed in Tables 1 and 2. Four strains (numbers 1422, 1902, 1560, and 1535) were Mexican *D. coccinea*, and one (2449) is a horticultural form. Numbers 1422 and 1535 are diploid (chromosome number $2n=32$) whereas 1902, 1560, and 2449 are tetraploid ($2n=64$). Data reported by Chin are included in the tables.[9]

The variation in the chromosome number from one strain to another does not show any constant variation either in the type or quantity of acetylenic substances present. One of the tetraploids (1902) is very much like the two diploids, especially in the tubers. The two other tetraploids contain abundant amounts of 1-phenylhepta-1:3-diyne-5-ene (IX), which is not

* Part II; FRANTZ KAUFMANN and JØRGEN LAM, *Acta Chem. Scand.* **21**, 311 (1967).

† Since the publication in *Chem. Brit.* **2**, 6 (1966), the name of this dahlia has been corrected to *Dahlia coccinea* Cav. var. *coccinea* (personal communication, E. R. H. Jones).

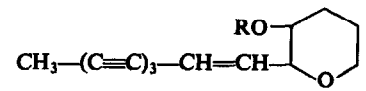
[1] E. E. SHERFF, *North Am. Flora* II, **2**, 45 (1955).
[2] F. KAUFMANN and J. LAM, *Acta Chem. Scand.* **19**, 1267 (1965).
[3] F. BOHLMANN and K.-M. KLEINE, *Chem. Ber.* **98**, 872 (1965).
[4] E. R. H. JONES, *Chem. Brit.* **2**, 6 (1966).

TABLE 1. CONSTITUENTS FROM THE TUBERS AND ROOTS*

	Dahlia coccinea		1422	1902	1560	1535	2449	Ref. 9
I	$CH_2{=}CH{-}(C{\equiv}C)_4{-}CH{=}CH{-}R$	R = CH_3	0·25	*t*	2·5	1	3	0·1
II		R = CHO	*t*			*t*		
III		R = CH_2OAc	*t*	*t*		*t*	*t*	0·5
IV		R = CH_2OH	*t*			*t*	*t*	3
V	$CH_2{=}CH{-}CH{=}CH{-}(C{\equiv}C)_3{-}CH{=}CH{-}R$	R = CH_3	*t*	0·03	17	0·1	6	
VI		R = CHO	3	6	*t*	1·5	8	
VII		R = CH_2OAc	5	24	?	*t*	4	1
VIII		R = CH_2OH	*t*	8		*t*	*t*	3
IX	$C_6H_5{-}(C{\equiv}C)_2{-}CH{=}CH{-}R$	R = CH_3			320	*t*	155	
X		R = CHO			28			
XI		R = CH_2OAc			74			
XII		R = CH_2OH			42			
XIII	$C_6H_5{-}(C{\equiv}C)_3{-}CH_3$				*t*	*t*	63	
XVI	$AcOH_2C{-}CH_2{-}CH(OAc){-}(CH{=}CH)_2{-}(C{\equiv}C)_3{-}CH_3$						10	
XVII	$HOH_2C{-}CH_2{-}CH(OH){-}(CH{=}CH)_2{-}(C{\equiv}C)_3{-}CH_3$						4	
XX	$CH_2{=}CH{-}CH{=}CH{-}(C{\equiv}C)_4{-}CH_3$							
XXI	$CH_2{=}CH{-}(CH{=}CH)_2{-}(C{\equiv}C)_3{-}CH_3$				*t*			
XXII	$CH_2{=}CH{-}CH{=}CH{-}CH{=}CH{-}(C{\equiv}C)_2{-}CH{=}CH{-}CH_3$				*t*	0·06		
XXIV	$CH_3{-}CH{=}CH{-}(C{\equiv}C)_2{-}(CH{=}CH)_2{-}R$	R = ?			*t*	*t*		

* mg per kg fresh wt.
t means amounts less than 0·02 mg/kg fresh wt.

TABLE 2. CONSTITUENTS FROM THE AERIAL PARTS*

	Dahlia coccinea		1560†	1535‡	2449‡	Ref. 9†
I	$CH_3—CH=CH—(C\equiv C)_4—CH=CH_2$				*t*	
IX	$C_6H_5=(C\equiv C)_2—CH=CH—R$	R = CH_3	900			
XI		R = CH_2OAc	48			
XII		R = CH_2OH	21			
XIV	$CH_3—(C\equiv C)_3—(CH=CH)_2—CH_2CH_2CH_2OAc$			5	*t*	0·5
XV	$CH_3—(C\equiv C)_3—(CH=CH)_2—CH_2CH_2CH_2OH$					30
XVI	$CH_3—(C\equiv C)_3—(CH=CH)_2—CH(OAc)CH_2CH_2OAc$			50		2·5
XVII	$CH_3—(C\equiv C)_3—(CH=CH)_2—CH(OH)CH_2CH_2OH$			215		
XVIII		R = $CH_3CO—$	120		*t*	2500
XIX		R = H—	60		*t*	200
XXIII	$CH_3—(C\equiv C)_3—(CH=CH)_2—(CH_2)_4—CH=CH_2$				*t*	

* Strains 1422 and 1902 showed no acetylenic compounds.
† mg per kg dry material.
‡ mg per kg fresh weight. *t* means amounts less than 0·02 mg.

found at all in either of the strains 1422 or 1902, and only in traces in the third one (1535). Whether the tetraploid forms have developed by autoploidy or alloploidy is not clear, but it seems likely to assume that the two tetraploids, 1560 and 2449, might be allotetraploid. The other tetraploid (1902) might be an autotetraploid.

The well-known ene-tetrayne-ene hydrocarbon (I) was isolated and characterized by u.v., i.r., and NMR spectra, and by thin-layer chromatography using authentic reference material. The corresponding aldehyde (II), acetate (III), and alcohol (IV) were found only in trace amounts. The ene-triyne-di-ene hydrocarbon (V) and its corresponding aldehyde (VI) are present in all the *D. coccinea* strains, whereas the corresponding acetate (VII) and alcohol (VIII) appear to be absent in the yellow *D. coccinea* (1560). The aldehyde was characterized by its u.v., i.r., and NMR spectra and by its similarity to the oxidation product of the corresponding alcohol with activated manganese dioxide.[5]

Of the remaining acetylenes in Tables 1 and 2, some are present in large amounts compared to the above-mentioned substances, but less widely distributed. 1-Phenylhepta-1:3-diyne-5-ene (IX) is the most abundant acetylene present in two of the tetraploid dahlias, 1560 and 2449. Coupling between all side-chain protons is observed in the NMR spectrum of IX. The methyl signal is split into two doublets ($J=7$ c/s and 2 c/s), which appear by coupling between the C-6 resp. C-5 olefinic proton. The coupling constant between the olefinic protons is about 17 c/s, which shows that the configuration of the double bond is *trans*.

In the yellow strain (1560), IX is found to be abundantly present in the tubers as well as in the aerial parts of the plant. It is seen from the tables that the corresponding acetate (XI) and alcohol (XII) are also present in fairly large amounts. The aldehyde (X) was isolated from the tubers of strain 1560 in an amount of 28 mg per kg fresh material. In the upper parts of the plant this aldehyde was not observed. Its presence was reported earlier[6] and subsequently Bohlmann, Köhn and Waldau[7] have isolated it from *Onopordon acanthium*.

The aldehyde (X) is crystalline (m.p. 59–61°) with a somewhat blurred u.v. spectrum: λ_{max} (ϵ) = 345 (12000); 319 (17700); 298·5 (16400); 280 (13800); 266·5 (17300); 254 nm (21·900). The i.r. spectrum shows the conjugated aldehyde group at 1675 cm^{-1}, the *trans* double bond at 948 cm^{-1} and the phenyl group at 1480, 1575, 750, 685 cm^{-1}. The acetylenic bands appear at 2220 cm^{-1}. Oxidation of the corresponding alcohol (XII) with manganese dioxide gives a product identical with the natural aldehyde. In the NMR spectrum of X the aldehyde proton appears at $\delta=9{\cdot}61$. The signal is split into a double doublet ($J=5{\cdot}0$ c/s and 2·3 c/s) due to coupling between the aldehyde proton and the two olefinic protons.

In the horticultural strain (2449) the hydrocarbon IX only was found to be present, and only in the tubers and roots. Furthermore this dahlia contains a fairly large amount of 1-phenylhepta-1:3:5-triyne (XIII). The two hydrocarbons are present in traces in only one of the diploid strains (1535).

The two hydrocarbons are not easily separated on ordinary silica gel thin-layer plates, but when caffeine is added to the silica gel (Fig. 1), the two substances may be easily separated.[8] After rechromatography in the same way, the two compounds can be separated completely. Due to different chemical shifts of the methyl protons in the propynyl group ($\delta=2{\cdot}00$) and the propenyl group ($\delta=1{\cdot}8$), the relative amounts of 1-phenylhepta-1:3:5-triyne and 1-

[5] J. ATTENBURROW, A. F. B. CAMERON, J. H. CHAPMAN, R. M. EVANS, B. A. HEMS, A. B. A. JANSEN and T. WALKER, *J. Chem. Soc.* 1104 (1952).

[6] J. LAM and F. KAUFMANN, Section paper 4–21: Chemical Constituents of the Genus *Dahlia* (Compositae).

[7] F. BOHLMANN, S. KÖHN and E. WALDAU, *Chem. Ber.* **99**, 3201 (1966).

[8] J. LAM and A. BERG, *J. Chromatog.* **20**, 168 (1965).

phenylhepta-1:3-diyne-5-ene have been estimated to be 2:1 on basis of the integrated NMR spectrum of the hydrocarbon mixture.

Most of these compounds have previously been reported by Jones[4] from various species, e.g. *D. coccinea*, and various garden varieties of other species. Furthermore the tetrahydropyranyl compounds (XVIII and XIX) have also been reported as constituents of *D. coccinea* L.[4,9,10] In this species the acetate (XVIII) and the corresponding alcohol (XIX) are present abundantly in the upper parts. We have also isolated these compounds from the yellow strain (1560) in fairly reasonable amounts, and in traces from a red tetraploid strain (2449) likewise from the upper parts of the plants, but there is no indication of these compounds in the other three strains, while the isomeric diol (XVII) is abundantly present in one of the diploids (1535) in the leaves and flowers.

The corresponding di-acetate (XVI) is also present in a relatively great amount in 1535. The diol is the most polar acetylenic compound from this dahlia. It is eluted in good yield and in a fairly pure state at the end of the development on the column, when acetone is used.

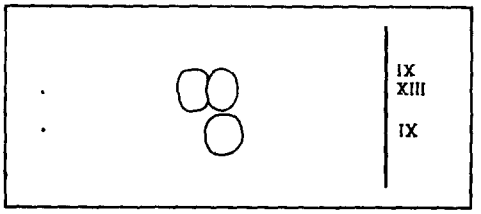


FIG. 1. THIN-LAYER CHROMATOGRAM OF A MIXTURE OF IX AND XIII ON A SILICA GEL PLATE IMPREGNATED WITH CAFFEINE. THE CHROMATOGRAM WAS DEVELOPED WITH LIGHT PETROLEUM TWICE.

The corresponding ketone (XXV) synthesized by oxidation of the diol (XVII) with activated manganese dioxide and the di-acetate made from the diol show data in agreement with the authentic substances as to the spectra (u.v., i.r., and NMR) and on thin-layer chromatography.

$$CH_3{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}\overset{\overset{O}{\|}}{C}{-}CH_2CH_2OH \qquad \text{(XXV)}$$

After total acetylation, the NMR spectrum of the diol shows that the alcohol proton signals disappear, and two unsplit methyl signals of the ester groups formed appear close to the signal of the $CH_3{-}C{\equiv}C$ group.

The remaining substances XIV, XV, XX, XXI, XXII, XXIII, and XXIV (several compounds) are more or less sporadically distributed, but too small amounts were present for a complete characterization in many cases.

In addition, there is evidence of the presence of other compounds in strain 1535, for instance (XXVI). In the upper parts of 1422 and 1902 no acetylenic compounds are detected, while cosmene[11] is present in 1422 and 1535.

$$CH_3{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}\underset{\underset{OR}{|}}{CH}{-}CH_2CH_2OR' \qquad \text{(XXVI)}$$

$$R{=}H,\ R'{=}OCCH_3 \text{ or } R{=}OCCH_3,\ R'{=}H$$

[9] C. G. CHIN, E. R. H. JONES, V. THALLER, R. T. APLIN, L. J. DURHAM, S. C. CASCON, W. B. MORS and B. M. TURSCH, *Chem. Comm.* 152 (1965).
[10] C. G. CHIN, Thesis, The Dyson Perrins Laboratory, Oxford, England (1965).
[11] J. S. SÖRENSEN and N. A. SÖRENSEN, *Acta Chem. Scand.* **8**, 284 (1954).

The compounds I–VIII are found to be frequently present in *Coreopsis*, *Cosmos*, *Bidens*, and *Dahlia*, which are genera all belonging to the group Heliantheae.[11–13] The compounds IX–XIII are also often found within the same group of Heliantheae. On the other hand it is evident that within the genus *Dahlia*, even within *D. coccinea*, there are great variations in the nature of the substances present.

MATERIAL AND EXPERIMENTAL

Our plant material was collected in Mexico and Guatemala by J. G. Hawkes, J. P. Hjerting, and R. Lester, and brought to Denmark by J. P. Hjerting. It was propagated in the Botanical Gardens of Copenhagen and Aarhus. Dried specimens of the material have been submitted to E. E. Sherff (Wisconsin) for determination, and herbarium material corresponding to the examined collections are deposited at the Botanical Institute, University of Aarhus. The chromosome number determinations were carried out by Dr. Peter Jacobsen, Copenhagen.

The collecting dates for the *Dahlia coccinea* investigated were the following:

1. *D. coccinea* Cav. (1422), Mexico, Querétaro State, road from San Juan del Rio–Querétaro highway north to Bernal, Cerro Galeras, east of road. Sept. 9, 1958. 2050 mts. Amongst shrubs on north-facing slope. Flowers flame-coloured. Chromosome number $2n=32$. 2. *D. coccinea* Cav. var. *coccinea* (1902). Guatemala, Dept. Sacatepéquez, above Santa María de Jesús, north-facing slope of Volcán de Agua, by trail. Hedges and bushy places by trail side. Oct. 7, 1958. 2250 mts. Flowers light orange-red, yellow disc. Plant to 50 cm. Chromosome number $2n=64$. 3. *D. coccinea* Cav. var. *coccinea* (1560). Mexico, Michoacán State, near Morelia, Cerro Punguato, grassy hill slopes. Sept. 26, 1958. 2200 mts. Flowers yellow, to 5 cm dia. Chromosome number $2n=64$. 4. *D. coccinea* Cav. (varietate *palmeri* adpropinquans) (1535). Mexico, Michoacán State, near Chilchota, road from Morelia to Guadalajara between Carapan and Tanguancicuaro. Sept. 21, 1958. 1600 mts. Rocky hillsides on south side of road. Flowers scarlet. Chromosome number $2n=32$.

The material examined was usually prepared for extraction immediately after harvesting, whether it was tubers and roots or flowers and leaves. Tubers were sliced and dropped into a mixture of dry ice and light petroleum (b.p. below 50°) and stored cold for extraction. The extraction was carried out with light petroleum, and repeated with a mixture of light petroleum and ether until the extract showed no acetylenic material after inspection of the u.v. spectrum. The extraction of the leaves was also carried out with light petroleum. Ether was avoided if a preliminary ether extraction did not show the presence of very much acetylenic material in

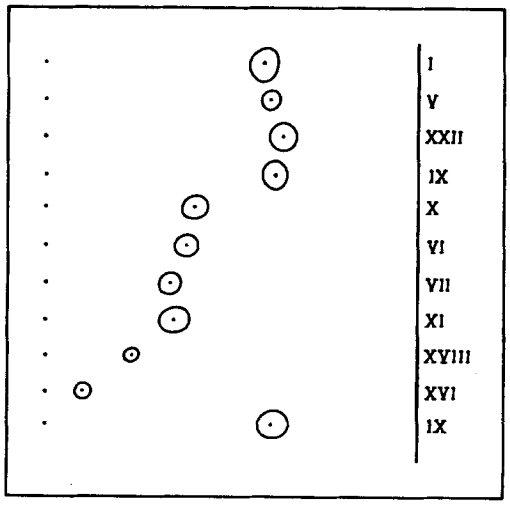


FIG. 2. THIN-LAYER CHROMATOGRAM OF ACETYLENIC HYDROCARBONS AND THE ACETATES. THE PLATE WAS PREPARED WITH SILICA GEL, AND DEVELOPED WITH ETHER IN LIGHT PETROLEUM (1:4).

[12] N. A. SÖRENSEN, in *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 219. Academic Press, London (1963).

[13] F. BOHLMANN, *Fortschr. chem. Forsch.* Band 6, Heft 1; *Naturstoffe* **65** (1966).

the extract (u.v. spectra), since the leaves contain larger amounts of ether extractible chlorophyll that might make subsequent separation difficult. Combined extracts were dried (Na_2SO_4), concentrated in a rotation evaporator, and transferred to a silica gel column for preliminary separation. The eluted fractions were inspected by u.v. spectroscopy. The fractions showing spectra corresponding to acetylenic compounds were compared on TLC (silica gel), and fractions with substances having the same R_f values were combined and rechromatographed either by column or by TLC. For the development of the columns as well as for the development on TLC plates, light petroleum or a mixture of light petroleum and ether was used (Figs. 1, 2, 3). The most polar substances were not easily eluted from the columns, even when ether was used. Thus, acetone was used for the final elution from the column. For characterization, u.v., i.r., and NMR spectroscopy were used.

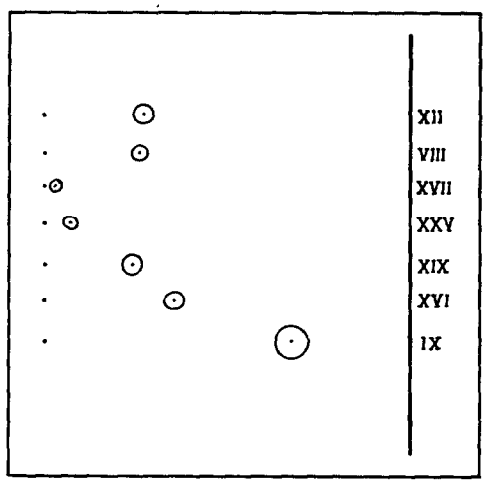


FIG. 3. THIN-LAYER CHROMATOGRAM OF THE ALCOHOLS. THE PLATE WAS PREPARED WITH SILICA GEL, AND DEVELOPED WITH ETHER IN LIGHT PETROLEUM (3:2).

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