Phytochemistry, Vol. 26, No. 11, pp. 3041–3045, 1987.
Printed in Great Britain.

0031–9422/87 $3.00+0.00
Pergamon Journals Ltd.

LATEX CONSTITUENTS OF *EUPHORBIA CANDELABRUM*, *E. GRANTII*, *E. TIRUCALLI* AND *SYNADENIUM GRANTII*

B. Uzabakiliho, C. Largeau*† and E. Casadevall*

Université Nationale du Rwanda, Crafop, B.P. 56, Butare, Rwanda; *Laboratoire de Chimie Bioorganique et Organique Physique, UA CNRS 456, E.N.S.C.P. 11 Rue Pierre et Marie Curie, 75231 Paris Cedex 05, France

(*Received* 12 *February* 1987)

Key Word Index—*Euphorbia candelabrum, E. grantii, E. tirucalli, Synadenium grantii, Euphorbiaceae*, analysis of latex; polyisoprenes; waxes; carboxylic acids; alkanes; triterpenoids; antioxidant.

Abstract—Quantitative and qualitative analyses were carried out on the latex of four *Euphorbiaceae* naturally growing in eastern Africa. Rubber (*cis*-1,4 linked units) was the only polyisoprene detected in these latices. However large differences in rubber levels were noted with the species and the latex origin. Waxes, mainly long chain ketones, are always minor constituents of the latices. Due to large variations in solubility, probably related to differences in rubber average molecular weight (maximal in *Synadenium grantii* and minimal in *Euphorbia grantii*), rubber was more or less efficiently separated from contaminant waxes. Acetone soluble products (resins) are the major constituents of the latices and chiefly comprise unsaponifiable compounds. In the case of *S. grantii* the acetone extract was dominated by triterpenoid compounds (ketones, alcohols, polyfunctional) with euphol, tirucallol and euphorbol as major constituents. The latex also contains small amounts of *n*-alkanes whose distribution differs sharply from that of cuticular hydrocarbons. The saponifiable fraction of the acetone extract contains *n*-fatty acids with the usual distribution and aromatic acids. Ionol, an antioxidant which could play an important role in the stabilization of *S. grantii* latex, was also detected.

INTRODUCTION

Three main features led to most of the previous studies on latex constituents in *Euphorbiaceae*. Firstly, such latices sometimes contain skin irritant and carcinogenic diterpene derivatives, especially phorbol esters [1–7]. Secondly, their triterpenol distribution proved to be useful in chemotaxonomy [8, 9]. Thirdly, the occurrence, in addition to polyisoprenic hydrocarbons, of fairly large amounts of highly reduced products in some latex-bearing plants suggested they may provide a renewable source of feedstocks and/or fuels [10–14]. In this connection, some species of the genus *Euphorbia*, growing in semi-arid lands of various regions, seemed especially interesting. Nevertheless few studies including both quantitative and structural information, were reported on the latex constituents of *Euphorbia* and other genera of *Euphorbiaceae*.

In the present work we examined the latex of four *Euphorbiaceae* naturally growing in Rwanda (eastern Africa): *Euphorbia candelabrum* Trem. ex Kotschy, *E. grantii* Oliver, *Synadenium grantii* Hook f and *E. tirucalli* L. (some latices of different origins were also studied for the latter species). The relative abundance and the composition of the main groups of constituents were determined in these latices. The efficiency of polyisoprene and wax separation was checked by spectroscopic methods, which also allowed the structure elucidation of the polyisoprenic hydrocarbons. In addition, analyses were carried out on the unsaponifiable and saponifiable constituents of the acetone extract of *S. grantii* latex.

RESULTS AND DISCUSSION

Fresh latices were submitted to successive extractions by acetone and dichloromethane; the two extracts were further separated into saponifiable and unsaponifiable products for that of acetone and into 'rubber' and 'waxes' fractions for the dichloromethane extract. A large content of dry matter was noted for the latices of the naturally growing plants (Table 1). Previous studies indicated dry weights ranging from 10 to 40% [15] for various species of *Euphorbiaceae* and a maximal value of *ca* 28% for *E. tirucalli* latex. In the four species examined, latices were dominated by acetone soluble constituents, mostly unsaponifiable. The relative abundances of the 'rubber' fraction and of the residue largely varied with the considered species and also, for *E. tirucalli*, with latex origin. 'Rubber' levels of 1–14% were previously reported for *E. tirucalli* latex [10, 16], but the purity of this fraction was not systematically checked while, as shown below, the fairly important 'rubber' fraction isolated from *E. tirucalli A* latex was exclusively composed of polyisoprenes. 'Waxes' were always minor constituents and the 'rubber' fraction was more important except for *E. grantii*. The residues generally contained a substantial level of ash and most of their constituents were water-soluble with the exception of *E.* candelabrum. The residue was particularly important for the latex of cultivated *E. tirucalli*. In fact, when compared with naturally growing plants, the former exhibited lower levels of dry matter and of the different extracts, the decrease being especially important for the 'rubber fraction'.

The present results, added to some previous observations [8, 10, 15, 17], suggest that most *Euphorbiaceae*

---

†Author to whom correspondence should be addressed.

Table 1. Main groups of constituents of the latex of *E. tirucalli*, *E. candelabrum*, *E. grantii* and *S. grantii**

Sample	Origin of the latex†	Dry weight‡	Acetone extract §, ‖		CH_2Cl_2 extract §, ¶		Residue §		
			Unsaponifiable	Saponifiable	'Rubber'	'Waxes'	Total	Ashes	Water soluble
E. tirucalli	*A*	41.5	57.5	2.5	13.2	2.1	13.4	2.1	7.5
E. tirucalli	*B*	23	40	6	1.6	1	40		35
E. tirucalli	*C*		60	8	5	3	14		12
E. candelabrum	*A*	31	50.5	5.7	12.5	3.7	8.9	0.5	1.6
E. grantii	*A*	41	52.3	4.5	2.5	4.1	34.3	10	24.2
S. grantii	*A*	35.5	49.2	16.3	6.9	1.7	15	3.7	11.4

*Average values from three expeirments.

†*A*: Fresh latex from plants naturally growing in Rwanda; *B*: fresh latex from greenhouse grown plants; *C*: dry latex from plants naturally growing in Brazil.

‡Total amount of dry matter, as % of the fresh latex.

§The abundance of the different fractions and of the residues was determined as % of the dry matter of the latex.

‖The crude acetone extracts contained a small amount of water soluble substances (respectively 4.7, 8, 2.5 and 2 % for *E. tirucally A*, *E. candelabrum*, *E. grantii* and *S. grantii*. In addition the extract of *S. grantii* contained *ca* 7 % of products insoluble in ether. The latter constituents and the water soluble ones were eliminated before saponification.

¶'Rubber' and 'waxes' fractions separated by hot alcohol (see below).

latices seem to be characterized by the predominance of acetone soluble unsaponifiable constituents and by a quite low contribution of 'waxes' and 'rubber' fractions. However, an important rubber level, *ca* 30 %, has been obtained in the latex of *E. characias* growing in the south of France (Casadevall, E., unpublished observation).

Chemical constitution of the dichloromethane extracts

The ^{1}H NMR spectrum of the crude extracts, obtained from the latex of naturally growing *E. candelabrum*, *E. grantii*, *E. tirucalli* and *S. grantii*, revealed the presence of polyisoprenes and waxy constituents (see below for the NMR characteristics of these groups). Two methods of separation, based on their supposed differences in solubility, were tested. The first one (precipitation of the 'rubber' fraction by the addition of various amounts of acetone to a dichloromethane solution) was not satisfactory, since an important part of the waxy constituents was also precipitated. On the contrary the second method (dissolution of the 'waxes' by hot alcohol from the dried extract) provided, at least in the case of *E. candelabrum*, *E. tirucalli* and *S. grantii*, a 'rubber' fraction containing no contaminant waxes. The latter method was applied to the four extracts and the fractions were examined by ^{1}H NMR, ^{13}C NMR and IR spectroscopy.

Previous studies on natural polyisoprenes of latex-bearing plants, and comparison with synthetic polymers, allowed the determination of the spectroscopic features corresponding to differently linked isoprene units (*cis* and *trans*-1,4; -1,2 and -3,4) [18–23]. *Hevea brasiliensis* latex was thus shown to exclusively contain -1,4-polyisoprene with at least 99 % of *cis*-units. Rubber (*cis*-1,4-polyisoprene) was also detected, but at a markedly lower concentration, in the latex of various plants including *Euphorbia* [10–12]. On the other hand, while it seems less widespread, the *trans*-1,4 isomer (gutta or balata) was also observed in various latices [24–26]. Furthermore the concomitant occurrence of rubber and gutta was noted in several species [27, 28].

The spectroscopic features of the 'rubber' fraction isolated from *S. grantii* latex indicated it exclusively consisted of *cis*-1,4-polyisoprenes. Thus, the IR absorptions, especially in the 8–10 μ range where *cis* and *trans*-1,4 units can be differentiated, were identical to these of *H. brasiliensis* rubber. This similarity was confirmed by ^{1}H NMR and ^{13}C NMR spectra (Table 2) where no peaks corresponding to *trans*-1,4, -1,2 and -3,4 units, or to non-isoprenic impurities, were detected. Regarding the 'waxes' fraction its spectra indicated the presence of long $(CH_2)_n$-chains and keto groups [^{1}H NMR (60 MHz, CCl_4): δ0.9 (Me), 1.25 (CH_2 chains), 2.2 (CH–C=O); IR (CCl_4): ν_{max} 1705 (carbonyl), ν_{max} 720 (CH_2 chains)]. No ester functions were noted. Accordingly this fraction was probably chiefly composed of long chain normal ketones which are usual constituents of higher plant waxes [29]. No traces of contaminant rubber were detected in the 'waxes' fraction from *S. grantii* latex.

According to its spectroscopic features the 'rubber' fraction from *E. tirucalli* was also exclusively composed of *cis*-1,4-polyisoprenes (Table 2). The 'waxes' fraction contained, in addition to the long chain ketones already observed in *S. grantii*, a substantial amount of *cis*-1,4-polyisoprenes [^{1}H NMR peaks (250 MHz, CCl_4) at δ1.25 (CH_2 of ketones) and 1.63 (Me of rubber) showing a relative intensity of *ca* 1/1 in this fraction].

The polyisoprenes of the 'rubber' fraction from *E. candelabrum* latex also only comprised *cis*-1,4-units as shown by the IR and NMR spectra. But this fraction was not entirely soluble in the solvents and the concentration range suitable for IR and NMR spectroscopy. In fact the IR spectra from KBr pellets showed, in addition to the typical absorptions of rubber, the presence of non-waxy impurities with, in particular, a large absorption at 1600 cm^{-1}. The 'waxes' fraction of *E. candelabrum* latex was composed, as in the case of *E. tirucalli*, of a mixture of long chain ketones and *cis*-1,4-polyisoprenes (relative intensity of the δ1.25 and 1.63 signals of *ca* 1.2/1). Consequently *E. candelabrum* and *E. tirucalli* rubbers, contrary to those of *H. brasiliensis* and *S. grantii* were slightly soluble in hot alcohol.

E. grantii afforded a small 'rubber' fraction. However, the IR and NMR spectra showed the lack of a significant content of polyisoprenes in this fraction. In fact it chiefly

Table 2. ^{1}H NMR and ^{13}C NMR spectra of the polyisoprenes isolated from *S. grantii*, *E. tirucalli* and *E. candelabrum* latex

Sample	Solvent	Chemical shift* 1	2	3	4	5
^{1}H NMR†						
S. grantii	C_6D_6	2.215		5.3	2.215	1.78 (1.79)
	CCl_4	2(2)		5.1 (5.08)	2(2)	1.63 (1.67)
	CS_2	2.03		5.12	2.03	1.63 (1.62)
E. tirucalli *E. candelabrum*	C_6D_6	2.20		5.3	2.22	1.78
^{13}C NMR‡						
S. grantii	CCl_4/ $CHCl_3$	32.14 (31.8)	134.97 (134.8)	124.98 (124.6)	26.34 (26.3)	23.40 (22.9)

*δ ppm, TMS as internal standard, ^{1}H NMR 250 MHz, ^{13}C NMR 25.17 MHz. The carbons of the C_5 units and the corresponding H were identified with the following numbering:

$[-CH_2-C(Me)=CH-CH_2-]$.
1 2 5 3 4

†The bracketed values correspond to previous observations on *H. brasiliensis* rubber. Previous results on the ^{1}H NMR spectra of gutta and *H. brasiliensis* rubber showed that the Me groups of *cis* and *trans*-1,4-polyisoprenes exhibit different chemical shifts [20, 21] with resonance occurring at a higher field in the *trans*-isomer. This difference depends on the solvent and was maximal in benzene (δ1.79 for *cis*-units instead of 1.65 for *trans*-units). In addition, some influence of the solvent was noted for CH_2 and CH groups.

‡^{13}C NMR of gutta and *H. brasiliensis* rubber showed, in CH_2Cl_2, important differences in the resonance of carbons 1 and 5 (respectively δ39.3 and 31.8 for 1, 15.3 and 22.9 for 5) [22]. Large variations were also noted for 1, in polyprenols, depending on the stereochemistry of the linked-1,4-isoprene units: δ32.24 for two *cis–cis* units and 39.74 for two *trans–trans* units with $CDCl_3$ as solvent [53]. In addition, relaxation times were longer in the *trans*-isomer. Hence large peaks occurred in gutta relative to rubber [23]. The bracketed values were reported for *H. brasiliensis* rubber in CH_2Cl_2 [22]. In the present case a 1:1 mixture of $CHCl_3$ and CCl_4 was required to obtain an efficient solubilization of *S. grantii* rubber.

contained non waxy products, insoluble in solvents as is the 'rubber' fraction of *E. candelabrum*. These compounds showed, from the IR spectra taken on KBr pellets, large absorptions due to aromatic and hydroxyl groups. The 'waxes' fraction of *E. grantii* was composed of long chain ketones and of a minor amount of rubber (relative intensity of the δ1.25 and 1.63 peaks of *ca* 5/1).

Long chain ketones and the same polyisoprenes (exclusively *cis*-1,4) were thus detected in the dichloromethane extracts of the four latices. However, large quantitative differences were noted. The rubber content was fairly high for *E. tirucalli* and *E. candelabrum* latex (in the former there was a small underestimation resulting from the presence of contaminant rubber in the 'waxes' fraction. In *E. candelabrum* a precise estimation was difficult due to a low level of rubber in 'waxes' and to the presence of some impurities in the 'rubber' fraction. A still substantial amount of rubber was noted for the latex of *S. grantii* and a complete separation with waxy constituents was achieved. On the contrary, *E. grantii* latex was characterized both by a very low rubber content and a poor separation. Large differences in solubility in hot alcohol were noted for the different types of rubber (*S. grantii*: insoluble, *E. tirucalli* and *E. candelabrum*: slightly soluble, *E. grantii*: soluble). According to previous reports [10, 11, 16, 27, 30] rubber average molecular weight largely varies with the species considered, but it generally seems to be markedly lower in most plants, including *Euphorbia*, when compared with *H. brasiliensis*. The present observations are probably related to a large decrease in rubber average M_r from *S. grantii* to *E. grantii*.

Chemical constitution of the acetone extract from S. grantii *latex*

Only two previous studies, concerned with lectins and triterpenols, were reported on *S. grantii* latex components [9, 31]. Therefore we examined, in addition to the 'rubber' and 'waxes' fractions, the acetone soluble compounds which are the major constituents of this latex. The acetone extract was first separated into unsaponifiable and saponifiable products (Table 1).

The predominant unsaponifiable compounds were further separated by column chromatography (Table 3). The first eluted subfraction (1) was minor and consisted of C_{14}–C_{19} *n*-alkanes with a maximum at C_{16} and no odd predominance. Low levels of hydrocarbons were recently observed in *E. candelabrum* and *E. tirucalli* latex [32] but their chemical structure was not established; small amounts of C_{18} and C_{20} branched hydrocarbons were also detected [33] in *H. brasiliensis* latex. *n*-Alkanes are commonly observed in higher plants; they are mainly located in cuticle and account for a low percentage of the whole organism. Such cuticular hydrocarbons are characterized by the dominance, or the exclusive occurrence, of odd compounds and generally show a maximum in the C_{21}–C_{33} range [29, 34]. *n*-Hentriacontane is the principal hydrocarbon identified from *E. tirucalli* stems [35] and the cuticular hydrocarbons of different biotypes of *E. esula* are dominated by C_{29}, C_{31} and C_{33} *n*-alkanes [36]. We therefore examined the hydrocarbons of *S. grantii* cuticle. They corresponded to an homologous series of C_{13}–C_{33} *n*-alkanes with a bimodal distribution

Table 3. Analysis of the acetone extract of *S. grantii* latex

Isolated fractions and subfractions*	Nature and relative abundance (%) of major constituents‡
Unsaponifiable subfractions†	
1 (0.6)	*n*-Alkanes: C_{14} (11.9), C_{15} (28.8), C_{16} (30.5), C_{17} (16.6), C_{18} (8.6), C_{19} (3.6)
2a (3.3)	Triterpenic ketones
2b (34)	Tetracyclic triterpenols: euphol (59), tirucallol (33), euphorbol (8)
3 (3.2)	Polyfunctional (OH and keto) triterpenoids
4 (16.5)	Sugars
Saponifiable fraction (25)	*n*-Fatty acids: $C_{12:0}$ (3.1), $C_{14:0}$ (3.3), $C_{16:0}$ (20.3), oleic (31.3) Aromatic acids: benzoic acid (1.7), phenylacetic acid (36.1), ionol (4.2)

*The values in parenthesis correspond to the abundance (%) of the different unsaponifiable subfractions and of the saponifiable fraction relative to the purified (see note ǀ, Table 1) acetone extract.

†Isolated from the unsaponifiable fraction by elution with hexane (1), Et_2O–ethyl acetate, 24:1 (2a and 2b), Et_2O–ethyl acetate, 1:1 (3) and MeOH (4).

‡Identifications were carried out by IR and NMR spectroscopy, GC and GC/MS with reference compounds. The identified constituents, or groups of constituents, account for *ca* 80% of the acetone extract. The figures in parenthesis correspond to the relative abundances (%) calculated for the constituents of subfraction (1), subfraction (2b) and for the saponifiable fraction.

(maxima at C_{15} and C_{31}) and a pronounced odd/even predominance, especially in the C_{26}–C_{33} range. Important differences in distribution were observed between *S. grantii* cuticular and latex hydrocarbons: lack of very long chain ($\geq C_{20}$) products and no odd dominance in the latter. Accordingly the biosynthesis of these two types of hydrocarbons probably takes place in distinct sites. Cuticular hydrocarbons of higher plants are generally considered to be formed in epidermal cells [37–39]. On the other hand, several types of biosynthetic activities, including rubber and triterpenol formation, were observed in different latices [15, 40–48].

Elution with ethyl acetate-diethyl ether allowed the recovery of about 40% of the total acetone extract (Table 3). Subfraction 2a consisted of triterpenic ketones while 2b was composed of tetracyclic triterpenols with a very large dominance of three constituents: euphol, tirucallol and euphorbol. This is consistent with a previous report, on the occurrence of the above compounds with two lanosterol isomers in *S. grantii* latex [9]. However, important differences were noted regarding relative abundances, since lanosterol isomers were predominant in the latter case. Subfraction 3 is a complex mixture of polyfunctional (hydroxy and keto) triterpenoids. The presence of oxo and hydroxysterols was noted in *E. fischeriana* [49]. According to its IR and NMR spectra the highly polar subfraction 4 was chiefly composed of sugars.

Three types of constituents were identified (Table 3) in the saponifiable fraction of the acetone extract. The first consisted of even normal fatty acids from C_{12}–C_{18} with oleic acid predominating; no traces were detected of odd acids or of very long chain products ($\geq C_{20}$). The exclusive occurrence, or the predominance, of even fatty acids is a common feature and, in higher plants, the most commonly observed are $C_{16:0}$ and $C_{18:1}$ fatty acids [29]. This is generally considered as the cause of the odd dominance in hydrocarbons, the latter being formed by decarboxylation of fatty acyl derivatives [50]. However, in *S. grantii* latex, an unexpected distribution with no odd predominance was noted for hydrocarbons while fatty acids are characterized by a classical distribution. The bulk of this acid fraction is probably esterified with triterpenols in the latex [17, 51] and is not implicated in hydrocarbon biosynthesis.

The second group of compounds was composed of two aromatic acids and phenylacetic acid which was the most important product of the saponifiable fraction.

Ionol (1,3-ditertiobutyl-2-hydroxy-5-methyl-benzene) was recovered in the saponifiable fraction due to the acidity of the phenol group. Ionol is known as an antioxidant agent; on the other hand it is well documented, firstly, that oxidation processes (action of phenol oxidases) play a major role in the coagulation of *H. brasiliensis* latex [52] and, secondly, that antioxidant constituents of the latex control these processes. Thus, ionol probably operates as a stabilizing agent in the latex of *S. grantii*.

This study on the latex composition in *E. candelabrum*, *E. grantii*, *E. tirucalli* and *S. grantii* shows that rubber (*cis*-1,4 units) is the only polyisoprene occurring in these latices. Large variations in rubber level were noted with the origin of the latex and the species. Important differences in solubility were also observed, hence a more or less efficient separation could be achieved of rubber and of the waxy constituents (long chain ketones) occurring in the dichloromethane extract. Examination of the acetone extract of *S. grantii* latex indicated that triterpenoids, especially triterpenols, are the major constituents. In addition several groups of products were identified, including *n*-alkanes with an unusual distribution, fatty and aromatic acids and ionol.

EXPERIMENTAL

Samples. Latices were collected around Butare, during the dry season, from naturally growing plants. The tapped plants were about 5 m high. Latex of *E. tirucalli* was also obtained from stems (*ca* 1 cm in diameter) of young plants grown at 28° under glass in the Station Centrale de Bioclimatologie de l'Inra (Versailles). Latex dry wt was determined after vacuum drying.

Extraction of fresh latices. Me_2CO soluble constituents were obtained by five successive extractions (stirring at room temp. for 15 min with 100 ml Me_2CO for 40 g of fresh latex) and the insoluble residue recovered by centrifugation. A similar method was applied to this residue, to obtain the CH_2Cl_2 soluble compounds and the final residue.

The CH_2Cl_2 extracts were separated into a 'rubber' and 'waxes' fraction by dissolving the latter in hot EtOH (four successive reflux for 4 hr with stirring, 80 ml of EtOH for 1 g of extract).

The crude Me_2CO extracts were purified by successive elimination of water soluble and Et_2O insoluble constituents. The resins so obtained were saponified (1 g of resins dissolved in 100 ml MeOH and 10 ml toluene, added to 1 g KOH dissolved in

H_2O, reflux for 3 hr under a N_2 atmosphere). The unsaponifiable and saponifiable products were respectively recovered by successive extractions with Et_2O under basic (pH 10) and acidic (pH 1) conditions. The unsaponifiable compounds were further fractionated on activity II neutral alumina. Saponifiable products were analysed after esterification of the acids by MeCOCl–MeOH.

NMR spectra. These were obtained, with TMS as int. standard, at 250 MHz for ^1H NMR and 25.17 MHz for ^{13}C NMR spectra.

GC analysis. This was carried out using a 25 m, 0.32 mm i.d., WCOT SE 52 capillary column, programmed from 100 to 250° at 4°/min for hydrocarbons and 170 to 250° at 2°/min for triterpenoids and saponifiable products (FID, He at 25 ml/min).

GC/MS. This was carried out (25 m, 0.23 mm i.d., WCOT CP-Sil 5 capillary column; temp. programme as above) with electron impact at 70 eV and an ion source temperature of 150°. Spectra were recorded from 30 to 450 mass units at a speed of 1 msec/a.m.u.; data were processed with Nermag–Sidar software.

Acknowledgements—This work was supported by a PNUD-UNESCO grant to B.U. We also thank, Dr S. De Parcevaux (INRA) for providing *E. tirucalli B* and *C* latex samples, Dr N. Sellier and Mrs J. Mauroy (ENSCP) for GC/MS technical assistance.

REFERENCES

1. Hecker, E. (1968) *Cancer Res.* **28**, 2338.
2. Evans, F. J. and Schmidt, R. J. (1976) *Phytochemistry* **15**, 333.
3. Fürstenberger, G. and Hecker, E. (1977) *Tetrahedron Letters* **11**, 925.
4. Kinghorn, A. D. (1980) *J. Pharm. Sci.* **69**, 1446.
5. Abo, K. A. and Evans, F. J. (1982) *Phytochemistry* **21**, 725.
6. Lin, L. J. and Kinghorn, A. D. (1983) *Phytochemistry* **22**, 2795.
7. Fuerstenberger, G. and Hecker, E. (1985) *Naturforschung* (C) **40**, 631.
8. Ponsinet, G. and Ourisson, G. (1965) *Phytochemistry* **4**, 799.
9. Nielsen, P. E., Nishimura, H., Liang, Y. and Calvin, M. (1979) *Phytochemistry* **18**, 103.
10. Nielsen, P. E., Nishimura, H., Otvos, J. W. and Calvin, M. (1977) *Science* **198**, 942.
11. Buchanan, R. A., Cull, I. M., Otey, F. H. and Russell, C. R. (1978) *Economic Botany* **32**, 131.
12. Buchanan, R. A., Cull, I. M., Otey, F. H. and Russell, C. R. (1978) *Economic Botany* **32**, 146.
13. Nemethy, E. K., Otvos, J. W. and Calvin, M. (1981) *Pure Appl. Chem.* **53**, 1101.
14. Declerck, M., Smets, P., Smets, J. and Roman, J. (1985) in *Energy from Biomass* (Palz, W., Coombs, J. and Hall, D. O. eds) p. 310.
15. Ponsinet, G. and Ourisson, G. (1967) *Phytochemistry* **6**, 1235.
16. Viaud, P. and Teisseire (1979) *Caoutchoucs Plastiques* 181.
17. Groeneveld, H. W. (1976) *Acta Bot. Neerl.* **25**, 167.
18. Binder, J. L. and Ransaw, H. C. (1957) *Anal. Chem.* **29**, 503.
19. Chen, H. Y. (1962) *Anal. Chem.* **34**, 1134.
20. Chen, H. Y. (1962) *Anal. Chem.* **34**, 1173.
21. Golub, M. A., Fuqua, S. A. and Bhacca, N. S. (1962) *J. Am. Chem. Soc.* **84**, 4981.
22. Duch, M. W. and Grant, D. M. (1970) *Macromolecules* **3**, 165.
23. Schaefer, J. (1972) *Macromolecules* **5**, 427.
24. Buchanan, R. A., Swanson, C. L., Weisleder, D. and Cull, I. M. (1979) *Phytochemistry* **18**, 1069.
25. Paterson-Jones, J. C. (1983) *Biomass* **3**, 225.
26. Roth, W. B., Carr, M. E., Davis, E. A. and Bagby, M. O. (1985) *Phytochemistry* **24**, 183.
27. Schlesinger, W. and Leeper, H. M. (1951) *Ind. Eng. Chem.* **43**, 398.
28. Leeper, H. M. and Schlesinger, W. (1954) *Science* **120**, 185.
29. Tulloch, A. P. (1976) in *Chemistry and Biochemistry of Natural Waxes* (Kolattukudy, P. E., ed.) p. 236. Elsevier, Amsterdam.
30. Swanson, C. L., Buchanan, R. A. and Otey, F. H. (1979) *J. Appl. Polymer Sci.* **23**, 743.
31. Premaratna, A., Shadak Sharaswamy, M. and Nanjappa, S. (1981) *Indian J. Biochem. Biophys.* **18**, 32.
32. Destordeur, M., Ramaut, J. L. and Rossi, M. E. (1983) *Saussurea* **14**, 29.
33. Nishimura, H., Philp, R. P. and Calvin, M. (1977) *Phytochemistry* **16**, 1048.
34. Hemmers, H. and Gülz, P.-G. (1986) *Phytochemistry* **25**, 2103.
35. Gupta, R. K. and Mahadevan, U. (1967) *Indian J. Pharm.* **29**, 152.
36. Manners, G. D. and Davis, D. G. (1984) *Phytochemistry* **23**, 1059.
37. Kolattukudy, P. E. (1968) *Plant Physiol.* **43**, 375.
38. Cassagne, C. (1970) *C. R. Acad. Sci. Ser. D.* **270**, 3055.
39. Cassagne, C. and Cezard, J. F. (1972) *C.R. Acad. Sci. Ser. D* **275**, 2077.
40. Archer, B. L., Audley, B. G., Cockbain, E. G. and McSweeney, G. P. (1963) *Biochem. J.* **89**, 565.
41. Ribaillier, D., Cuong, T. C. and Fournier, P. (1964) *C. R. Acad. Sci.* **258**, 2218.
42. Chesterton, C. J. and Kekwick, R. G. O. (1968) *Arch. Biochem. Biophys.* **125**, 76.
43. Hepper, C. M. and Audley, B. G. (1969) *Biochem. J.* **114**, 379.
44. Lynen, F. (1969) *J. Rubber Res. Inst. Malays* **21**, 389.
45. Biesboer, D. D. and Mahlberg, P. G. (1981) *Am. J. Botany* **68**, 498.
46. Sipat, A. B. (1982) *Phytochemistry* **21**, 2613.
47. Nemethy, E. K., Skrukrud, C., Piazza, G. J. and Calvin, M. (1983) *Biochim. Biophys. Acta* **760**, 343.
48. Groeneveld, H. W., Furr, M. and Mahlberg, P. (1984) *Am. J. Botany* **71**, 123.
49. Schroeder, G., Rohmer, M., Beck, J. P. and Anton, R. (1980) *Phytochemistry* **19**, 2213.
50. Kolattukudy, P. E., Croteau, R. and Buckner, J. S. (1976) in *Chemistry and Biochemistry of Natural Waxes* (Kolattukudy, P. E., ed.) p. 290. Elsevier, Amsterdam.
51. Warnaar, F. (1981) *Phytochemistry* **20**, 89.
52. Brzozowska-Hanower, J., Hanower, P. and Lioret, C. (1978) *Physiol. Veg.* **16**, 231.
53. Ibata, K., Kageyu, A., Takigawa, T., Okada, M., Nishida, T., Mizuno, M. and Tanaka, Y. (1984) *Phytochemistry* **23**, 2517.