Phytochemistry, Vol. 28, No. 11, pp. 3159–3162, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00

TERPENOIDS AND AN APOCAROTENOID FROM SEEDS OF *BIXA ORELLANA*

ISAAC J. O. JONDIKO and GERALD PATTENDEN

Department of Chemistry, The University, Nottingham NG7 2RD, U.K.

(*Received in revised form* 19 *April* 1989)

Key Word Index—*Bixa orellana*; Bixeae; annatto; geranylgeraniol; δ-tocotrienol; apocarotenoids.

Abstract—*all-E*-Geranylgeraniol is present in the oleoresin from *Bixa orellana* to the extent of 57%, or approximately 1% of dry seeds which makes *B. orellana* the richest known source of this important C_{20}-terpene alcohol. Other compounds isolated for the first time from seeds of *Bixa* were the terpenes farnesylacetone, geranylgeranyl octadecanoate and geranylgeranyl formate, δ-tocotrienol, and an apocarotenoid.

INTRODUCTION

The commercial extract of seeds of *Bixa orellana*, known as 'annatto' is rich in the C_{24}-apocarotenoid bixin (**1**). Purified bixin is used in a number of formulations in the food industry [1], for example in colouring butter, cheese, ice-cream, bakery products and edible oils. Annatto has been shown to have pro-vitamin A activity [2], besides medicinal and cosmetic properties [3].

Bixin (**1**) was the first naturally occurring *cis*-carotenoid to be isolated from natural sources [4–6]. Although first isolated in 1875, it was not until 1961 that its full structure and stereochemistry were established largely through studies of its ^{1}H NMR spectral data [7]. The 9′-*cis* stereochemistry assigned to the pigment was later confirmed by synthesis [8]. Bixin belongs to the relatively small family of natural apocarotenoids whose formation is thought to occur by oxidative degradation of the parent C_{40}-carotenoids [9]. Other examples of apocarotenoids include crocetin [10], β-apo-10′-carotenal, [11], β-citraurin and abscisic acid [12, 13]. Although some circumstantial evidence supports the proposal that certain apocarotenoids are derived from degradative pathways, no detailed evidence pertaining to bixin (**1**) has been published. As a contribution to the understanding of the biosynthesis of bixin, we have carried out an examination of the terpenoids and the carotenoid content of fresh seeds of *Bixa orellana* [14].

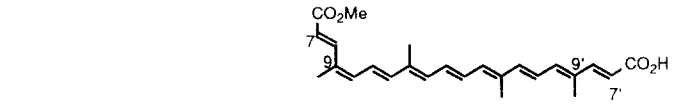
RESULTS AND DISCUSSION

Dry seeds of *Bixa orellana* were first extracted with hexane to produce an oleoresin, and then with methanolic–methylene dichloride to produce crystalline bixin (**1**) and a filtrate containing a mixture of other pigments and terpenoids. After concentration, the filtrate was extracted with hexane to obtain a hypophase and an epiphase. The principal constituent in the oleoresin was *all-E*-geranylgeraniol (**2**); a minor constituent was farnesyl acetone (**3**). Chromatographic analysis of the epiphase showed the presence of several components, from which geranylgeranyl octadecanoate (**4**), geranylgeranyl formate (**5**), δ-tocotrienol (**6**) and the diapo 8-oxo ester (**7**) were isolated following multiple elutions on HPLC.

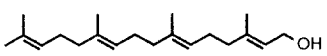
Quantitative studies demonstrated that *all-E*-geranylgeraniol (**2**) was present in the oleoresin from *Bixa* to the extent of 57%, or *ca* 1% of dry seeds. This makes *Bixa orellana* the richest known source of this important terpene alcohol. *all-E*-Geranylgeraniol, as its diphosphate, is the central intermediate in carotenoid biosynthesis, where it undergoes 'tail-to-middle' coupling to give prephytoene, the precursor to phytoene which is the first carotenoid in the Porter–Lincoln biosynthetic pathway to carotenoids. Geranylgeraniol has also been isolated from linseed oil and peanut oil [15], from *Cedrela toona* [16], bumble bees [17], *Phytophthora cactorum* [18] and ants [19], and from *Pterodon* species [20]. The structure and stereochemistry assigned to *all-E*-geranylgeraniol from *Bixa*, followed largely from inspection and comparison of its NMR spectral data with those described in the literature for both the natural [16] and the synthetic material [21].

Farnesylacetone (**3**), which is possibly a degradation product of geranylgeraniol in *B. orellana* has previously been isolated from *Sargassum micracanthum* [22], *Cecropia adenopus* [23], tomato [24], *Cannabis sativa* [25], burley tobacco [26] and *Carphephorus* species [27]. To our knowledge, geranylgeranyl formate (**10**) has not been reported in Nature before this study. Other natural terpene formates however, are quite well known. For example, essential oils of *Perlargonium roseum* contain formates of geraniol, citronellol and nerol [28], while the alarm pheromone of *Tyrophagus putrecentiae* is largely composed of neryl formate [29]. The structure of geranylgeranyl formate from *B. orellana* followed from comparison with an authentic sample synthesized from *all-E*-geranylgeraniol and acetic formic anhydride. Formate apart, other esters of geranylgeraniol are widely distributed in Nature. *all-E*-Geranylgeranyl octadecanoate (**4**) for example, was earlier reported in the essential oils of *Picea abies* [30]. Geranylgeraniol is also one of the alcohols esterifying bacteriophyll *a* of *Rhodospirillum rubrum* [31], and chlorophyll of newly formed leaves of *Aesculus hippocastanium* [32] and barley [33].

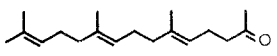
all-E-Geranylgeraniol is well-known as being involved in the natural prenylation of quinones, as demonstrated by the isolation of tocotrienols, viz **6**, from palm oil [34], *Hevea braziliensis* [33], *Sargassum* species [35] and some



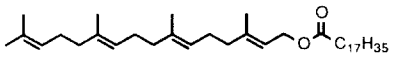
1



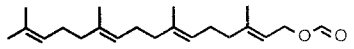
2



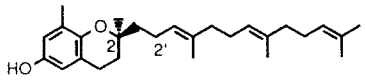
3



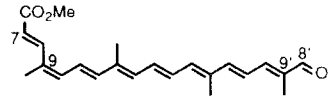
4



5



6



7

leguminous seeds [36]. Tocotrienols have been shown to influence settling of swimming larvae of *Coryne uchidae* [35], and to possess antioxidation properties [37]. The presence of δ-tocotrienol (**6**) in *B. orellana* may influence the stability of bixin against oxidation. The structure and stereochemistry assigned to δ-tocotrienol were deduced from comparative spectroscopic data with those reported in the literature [35].

Methyl 9′-*cis*-apo-1-bixinal ester (methyl 8-oxo-9′*cis*-8, 6′-diapocaroten-6′-oate) (**7**) is a new pigment to be isolated from *B. orellana*. It is possible that the metabolite is produced naturally by enzymic oxidative cleavage at C-7/C-8 in bixin. The structure of **7** followed from inspection of its light absorption and mass spectrometric fragmentation data, together with analysis of chemical shift and spin–spin coupling data in the ^{1}H NMR spectrum. Interestingly, the same diapo-oxo-ester (**7**) has been reported by Karrer *et al.* [38] and by Barber *et al.* [7] as one of the products resulting from oxidation of bixin by permanganate. The physical and spectral data for our natural product were closely similar to those described for the synthetic material.

Despite thorough and extensive chromatography, we were unable to establish the presence of higher carotenoids, i.e. C_{40}-, C_{32}-, analogues of bixin in seeds of *Bixa orellana*. During the course of our studies, however, Tirimanna [3] published tenuous evidence, using TLC comparisons, for the presence of β-carotene, lutein, and zeaxanthin in fresh seeds of *Bixa*.

EXPERIMENTAL

General. Dry seeds of *Bixa orellana* were obtained from the Kenya Cereal Board Authority . All solvents were purified by standard methods, and standard precautions against the effects of light, heat, acid, base, oxygen and peroxides were taken during the handling of coloured extracts from the *Bixa* seeds. HPLC separations were carried out on either Zorbax ODS, Zorbax Sil or Water's Pre Pak 500 Sil columns (UV and refractive index detectors), whereas GLC analyses were performed on glass columns packed with 3% OV-1 on chromosorb B (FID detector).

Extraction of seeds of Bixa orellana. Dry seeds (800 g) of *B. orellana* were shaken under dist. hexane (1.6 l) for 4 hr at 25°, and the hexane extract was then filtered off and evapd *in vacuo* to leave an oleoresin (12.5 g). The residual seeds were shaken under 1.6 l MeOH–CH_2Cl_2 (1:1) for 4 hr at 25°, and once again filtered. The filtrate was evapd *in vacuo* to half of its original vol. and the residue was then left at −10° overnight. Bixin (**1**) (11 g) separated, and was recrystallized from HOAc to give red-purple crystals, mp 195° (lit. mp 196°); UV $\lambda_{max}^{C_6H_6}$ nm (ε): 503 (11 500), 470 (12 500), 444 (83 400); IR ν_{max} cm^{-1} 3400 (*br*), 1720, 1660, 1600, 1385, 1300 and 900; ^{1}H NMR (250 MHz, $CDCl_3$): δ7.80 (1H, *d*, *J* = 15.8 Hz, H-7), 7.22 (1H, *d*, *J* = 15.8 Hz, H-7′) 6.30–7.00 (10H, *m*, 10 × :CH), 5.8 (1H, *d*, *J*=15.8 Hz, H-8) 5.68 (1H, *d*, *J* =15.8Hz, H-8′), 3.66 (3H, *s*, OMe), 1.8–2.0 (12H, *m*, 4 × :CMe). The filtrate remaining from the separation of bixin was concd. to 400 ml, and then extracted with hexane (4 × 400 ml) to produce an epiphase and a hypophase. Evapn of the solvents left a red solid (6.15 g) (from the epiphase), and a red paste (30 g) (from the hypophase), whose chemical constituents were examined separately.

Farnesylacetone (all-E-6,10,14-*tetramethylheptadeca*-5,9,13-*trien*-2-*one*) (**3**) and *geranylgeraniol* (all-E 3,7,11,15-*tetramethylhexadeca*-2,6,10,14-*tetraen*-1-*ol*) (**2**). A sample (3 g) of the oleoresin was distilled to give a colourless oil (1.6 g), bp 130–170°. Chromatography of the distillate on silica gel using increasing proportions of Et_2O in petrol (bp 40–60°) as eluant gave: (i) Farnesylacetone (**3**) (eluted first; 30% Et_2O) as a colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2930, 1717, 1665, 1440, 1390, 1140, ^{1}H NMR

(250 MHz, $CDCl_3$): δ1.62 (9H, *s*, 3 × :CMe), 1.70 (3H, *s*,:CMe), 1.96–2.10 (*br m*, $-CH_2-$), 2.40 (2H, *t*, *J*=7 Hz, CH_2CO), 2.12 (3H, *s*, Ac), 5.0–5.25 (3H, *m*, :CH); (*m/z* 262.2298. $C_{18}H_{30}O$ requires:[M]$^+$ 262.2297); MS *m/z* (rel.int.):262 (5), 219 (3), 205 (2), 193 (4), 191 (2), 179 (2), 152 (1), 151 (4), 139 (3), 137 (17), 125 (19), 123 (16), 111 (3), 110 (3), 84 (10), 83 (11), 71 (9), 69 (100), 57 (18) 55 (33): (ii) geranylgeraniol (**2**), IR $\nu_{max}^{CHCl_3}$ cm^{-1}:3600, 2930, 1665, 1440, 1390, 1140, 1095, 985, ^{1}H NMR (250 MHz. $CDCl_3$): δ5.45 (1H, *br t*, *J*=7 Hz, H-2) 5.13 (1H, *br m*, 3 × :CH), 4.15 (2H, *d*, *J*=7Hz, H-1), 1.9–2.20 (12H, *m*, $-CH_2-$), 1.62 (9H, *s*, 3 × : CMe), 1.7 (6H, *s*, : CMe), 1.51 (1H, *s*, OH); ^{13}C NMR (25.15 MHz, $CDCl_3$): δ16.0 (*q*, C-7 Me and C-11 Me), 16.3 (*q*, C-3 Me), 17.7 (*q*, C-15 Me), 25.7 (*q*, C-16), 26.4 (*t*, C-12), 26.7 (*t*, C-8) 26.8 (*t*, C-4), 39.6 (*t*, C-5), 39.7 (*t*, C-9 and C-13), 59.4 (*t*, C-1) 123.4 (*d*, C-2), 123.8 (*d*, C-6), 124.2 (*d*, C-10) 124.4 (*d*, C-14), 131.3 (*s*, C-15), 135.0 (*s*, C-11), 135.5 (*s*, C-7), 139.9 (*s*, C-3); (*m/z* 290.2612. $C_{20}H_{34}O$ requires [M]$^+$ 290.2609); MS *m/z* (rel.int.):290 (10), 272 (15), 247 (5), 229 (10), 221 (30), 204 (70), 203 (50), 173 (5), 161 (60), 136 (60), 117 (3), 85 (15), 69 (100).

The oleoresin was calculated to be 1.5% of the dry seeds of *Bixa*, while the distillate was found to be 52% of the oleoresin. Quantitative estimation of free geranylgeraniol in *Bixa* seeds was made through GLC analysis (3% OV-1 on chromosorb B, programmed 160–210°) using geraniol as an int. standard. This study showed that the oleoresin contained 57% geranylgeraniol, equivalent to 1% of the *Bixa* seeds.

Geranylgeranyl octadecanoate (all-E 3,7,11,15-*tetramethyl hexadeca*-2,6,10,14-*tetraenyl octadecanoate*) (**4**), *geranylgeranyl formate* (all-E 3,7,11,15-*tetramethylhexadeca*-2,6,10,14-*tetraenyl formate*) (**5**), *δ-tocotrienol* [3,4-*dihydro*-2,8-*dimethyl*-2 (4,8,12-*trimethyl*-3E,7E,11-*tridecatrienyl*)-2*H*-1-*benzopyran*-6-*ol*] (**6**) and *methyl* 8′-*oxo*-9-*Z*-8′,6-*diapocaroten*-6-*oate* (**7**). The components of the epiphase resulting from the extraction of *Bixa* seeds were separated by HPLC in $CHCl_3$ soln to give: (i) geranylgeranyl octadecanoate (eluted first), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1670, 1120, ^{1}H NMR (250 MHz, $CDCl_3$): δ5.35 (1H, *t*, *J* = 7 Hz, H-2), 5.14 (3H, *m*, 3 × : CH), 4.62 (1H, *d*, *J* = 7 Hz, H-1), 2.33 (2H, *t*, *J* = 7 Hz, CH_2CO), 2.0–2.2 (12H, *m*, 6× CH_2). 1.7 (6H, *s*, 2 ×: CMe), 1.61 (9H, *s*, 3 × : CMe), 1.26 (32H, *m*, 16 × CH_2), 6.9 (3H, *t*, *J* = 6 Hz, Et). (*m/z* 556.5215. $C_{38}H_{68}O_2$ requires: [M]$^+$ 556.5219); MS *m/z* (rel. int.): 556 (4), 487 (3), 459 (5), 297 (4), 289 (3), 284 (36), 283 (14), 272 (60), 267 (5), 259 (7), 239 (10), 229 (40), 203 (100), 202 (65).

(ii) Geranylgeranyl formate (eluted second) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1665, 915, ^{1}H NMR (250 MHz, $CDCl_3$) δ8.1 (1H, *s*, CHO), 5.4 (1H, *t*, *J*=7Hz, H-2), 5.12 (3H, *m*, 3 × : CH), 4.7 (2H, *d*, *J*=7Hz, H-1), 1.9–2.2 (12H, *m*, 6 × CH_2), 1.72 (3H, *s*, :CMe), 1.70 (3H, *s*, :CMe), 1.6 (9H, *s*, 3 × : CMe), (*m/z* 318.2554; $C_{21}H_{34}O_2$ requires:[M]$^+$; 318.2559); MS *m/z* rel.int):318 (7), 272 (6), 203 (11), 161 (14), 136 (57), 135 (41), 121 (36), 107 (36), 93 (78), 83 (25), 69 (100), 55 (64), 52 (21).

(iii) Methyl 8′-oxo-9 -*Z*-8′,6 -diapocaroten-6-oate (eluted third), which crystallized from MeOH as red crystals, mp, 145°C, UV $\lambda_{max}^{C_6H_6}$ nm (ε): 497 (84 000), 462 (100 000), 439 (70 000); IR $\nu_{max}^{CHCl_3}$ cm^{-1}:1710, 1665, 1610, 1380, 1080, 915. ^{1}H NMR (250 MHz, $CDCl_3$): δ9.46 (1H, *s*, H-8′) 7.97 (1H, *d*, *J* = 15.5 Hz, H-7), 6.97 (1H, *d*, *J*=15.5 Hz, H-12′), 6.3–7.0 (9H, *m*, 9 × :CH), 5.98 (1H, *d*, *J*=15.5 Hz, H-8), 3.97 (3H, s, OMe), 2.03 (3H, *s*, :CMe), 1.97 (3H, *s*, :CMe), 1.95 (6H, *s*, 2 ×:CMe); (*m/z* 352.2042; $C_{23}H_{28}O_3$ requires [M]$^+$ 352.2038); MS *m/z* (rel.int.): 352 (100), 196 (10), 159 (18), 143 (45), 132 (22), 119 (44), 91 (60), 79 (44) 69 (80), 68 (10).

(iv) *δ*-tocotrienol (eluted last), UV λ_{max}^{EtOH} nm (ε): 297 (2100), IR $\nu_{max}^{CHCl_3}$ cm^{-1}:3625, 2950, 1620, 1485, 1390, 1155. ^{1}H NMR (250 MH_z, $CDCl_3$): δ6.47 (1H, *d*, *J*= 2Hz, H-8), 6.38 (1H, *d*, *J*=2Hz, H-6), 5.0–5.2 (3H, *m*, 3 × : CH), 4.45 (1H, *br*, OH) 2.69 (2H, *t*, *J*=7Hz, H-4), 2.12 (3H, *s*, :CMe), 1.96–2.1 (12H, *m*, 6 × CH_2), 1.7 (2H, *q*, *J*=7Hz, H-2), 1.68 (3H, *s*, : CMe), 1.59 (9H, *s*, 3 × :CMe), 1.26 (3H, *s*, Me); ^{13}C NMR (25.15 MHz, $CDCl_3$): δ15.9 (*q*, C-8-Me), 16.0 (*q*, C-4-Me), 17.7 (*q*, C-12-Me), 22.2 (*t*, C-4-CH_2), 24.1 (*t*, C-11-CH_2), 24.6 (*q*, C-1-Me), 25.7 (*q*, C-13), 26.7 (*t*, C-9-CH_2), 26.8 (*t*, C-5-CH_2), 31.5 (*t*, C-3-CH_2), 39.8 (t, C-6-CH_2), 76.5 (*s*, C-1), 112.7 (*d*, C-5), 1 5.7 (*d*, C-7), 121.3 (*s*, C-4′), 124.3 (*d*, C-3), 124.4 (*d*, C-7), 124.5 (*d*, C-11), 127.4 (*s*, C-8), 131.2 (*s*, C-12), 135.0 (*s*, C-8), 135.2 (*s*, C-4), 146.0 (*s*, C-8′), 147.8 (*s*, C-6); (*m/z* 396.3042; $C_{27}H_{40}O_2$ requires: [M]$^+$ 396.3028); MS *m/z* (rel. int.): 396 (100), 219 (4), 204 (8), 192 (20), 177 (50), 175 (21), 163 (14), 137 (95), 121 (20), 109 (22), 45 (18), 69 (85), 41 (37). $[\alpha]_D^{20°}$ + 20.5° (EtOH;*c* 0.02).

Methyl bixin. A soln of the hypophase (1 g), from the extraction of *Bixa* seeds, in MeOH (15 ml) and C_6H_6 (15 ml) was added to a suspension of KOH pellets (0.5 g) in dry methyl acetate (30 ml). Dimethyl sulphate (0.5 g) was added to the suspension, and the mixture was then allowed to stand overnight. The mixture was evapd to dryness *in vacuo*, and the residue was then purified by chromatography on silica gel using CH_2Cl_2 – EtOAc (9:1) as eluant. The coloured fractions (λ_{max} 400 nm) were re-chromatographed on a Zorbax sil HPLC column, using hexane–EtOAc (9:1) as eluant to give:

(i) methyl 8-oxo-9′-*z*-8,6′-diapocaroten-6′-oate (eluted first), mp 140° (MeOH), which showed identical spectroscopic data to those described earlier, and

(ii) methyl bixin (eluted second), which crystallized from methanol as red crystals, mp 162° (lit. [8], mp 163°), UV $\lambda_{max}^{C_6H_6}$ nm (ε): 501 (109 900), 469 (124 000), 442 (84 000) IR $\nu_{max}^{CHCl_3}$ cm^{-1}:1700, 1660, 1280, 1125, 1000, 985, 900 and 860; ^{1}H NMR (250 MHz, $CDCl_3$): δ7.97 (1H, *d*, *J*=15.5 Hz, H-7), 7.4 (1H, *d*, *J* =15.5 Hz, H-7′), 6.3–7.0 (10H, *m*, 10× :CH), 5.93 (1H, *d*, *J* =15.5Hz, H-8), 5.89 (1H, *d*, *J*=15.5Hz, H-8′), 3.80 (3H, *s*, OMe), 3.79 (3H, *s*, OMe), 1.95–2.0 (12H, 4× :CMe). (*m/z* 408.2313; $C_{26}H_{32}O_4$ requires:M$^+$ 408.2301).

Geranylgeranyl formate (all-E-3,7,11,15-*tetramethylhexadeca*-2,6,10,14-*tetraenyl formate* (**5**). Acetic formic anhydride (9.35 g) was added to a stirred suspension of geranylgeraniol (0.5 g) and $NaHCO_3$ (0.3 g) under Et_2O (100 ml). The mixture was stirred at 25° for 1.5 hr and then filtered. The filtrate was evapd *in vacuo*, and the residue purified by chromatography on silica gel, using Et_2O–petrol (bp 60–80°) (9:1) as eluant, to give the formate (0.4 g, 80%) as a pale yellow oil. The formate showed identical spectroscopic data to those described above.

Acknowledgement—I. J. O. Jondiko thanks Professor T. R. Odhiambo (Director of International Centre of Insect Physiology and Ecology, Nairobi, Kenya) for a research scholarship.

REFERENCES

1. Dendy, D. A. V. (1966) *East Afr. Agric. Forest. J.* **32**, 126.
2. Santamaria, L., Martinez, M. L., Asenjo, C. F. (1965) *J. Agric. Univ. Puerto Rico* **49**, 259.
3. Tirimanna, A. S. L. (1981) *Mikrochimica Acta* II, 11.
4. Karrer, P., Helfenstein, A., Widmer, R. and Van Itallie, T. B. (1929) *Helv. Chim. Acta* **12**, 741.
5. Zechmeister, L. (1944) *Chem. Rev.* **34**, 267.
6. Zechmeister, L. and Lunde, K. (1955) *J. Am. Chem. Soc.* **77**, 1647.
7. Barber, M. S., Hardisson, A., Jackman, L. M. and Weedon, B. C. L. (1961) *J. Chem. Soc.* 1625.
8. Pattenden, G., Way, J. E. and Weedon, B. C. L. (1970) *J. Chem. Soc.* (C), 234.
9. Isler, O., Ruëgg, R. and Schudel, P. (1962) *Recent Progress in the Chemistry of Natural and Synthetic Colouring Matter*

and Related Fields (Gore, T. S., Joshi, B. S., Sunthankar, S. V. and Tilak, B. D., eds), p. 39. Academic Press, New York.
10. Pfander, H. and Schurtenberger, H. (1982) *Phytochemistry*, **21**, 1039.
11. Yokoyama, H. and White, M. J. (1966) *Phytochemistry* **5**, 1159.
12. Taylor, H. F. and Burden, R. S. (1970) *Phytochemistry* **9**, 2217.
13. Milborrow, B. V. (1972) *Biochem. J.* **128**, 1135.
14. Jondiko, I. J. O. (1983) Ph.D. Thesis, University of Nottingham.
15. Fedeli, E., Capella, P., Cirimele, M. and Jacini, G. (1966) *J. Lipid. Res.* **7**, 437.
16. Nagasampagi, B. A., Yankov, L. and Sukh, D. (1967) *Tetrahedron Letters* **2**, 189.
17. Svensson, B. G. and Bergsbrom, G. (1977) *Insectes Soc.* **24**, 213.
18. Richards, J. B. and Hemming, F. W. (1972) *Biochem. J.* **128**, 1345.
19. Loefqvist, J. and Bergsbrom, G. (1980) *J. Chem. Ecol.* **6**, 309.
20. Fascio, W., Mors, W. B., Gilbert, M. B., Mahajan, J. R., Monteiro, M. B., Santos, D. F. and Vichnewski, W. (1976) *Phytochemistry* **15**, 201.
21. Coates, R. M., Ley, D. A. and Cavender, P. L. (1978) *J. Org. Chem.* **43**, 4915.
22. Kusumi, T., Ishitsuka, M., Nomura, Y., Konno, T. and Kakisawa, H. (1979) *Chem. Letters* 1181.
23. Neidlein, R. and Koch, E. (1980) *Arch. Pharm. (Weinheim)* **313**, 199.
24. Buttery, R. G., Seifert, R. M. and Ling, L. (1969) *Chem. Ind. (London)* **8**, 238.
25. Hendriks, H., Malingre, T. M., Batterman, S. and Bos. R (1978) *Pharm. Week Bl.* **113**, 413.
26. Takane, F., Reiko, K., Hajime, M., Hajime, K. and Masao, M. (1976) *Agric. Biol. Chem.* **40**, 303.
27. Karlsson, K., Wahlberg, I. and Enzell, C. R. (1972) *Acta. Chem. Scand.* **26**, 2839.
28. Calvarano, M. (1966) *Essen. Derative Agrum.* **36**, 100.
29. Kuwahara, Y., Ishii, S. and Fukami, H. (1975) *Experientia* **31**, 1115.
30. Rainer, E. (1980) *Phytochemistry* **19**, 321.
31. Brockmann, H., Knobloch, G., Schweer, I. and Trowitzch, W. (1973) *Arch. Mikrobiol.* **90**, 161.
32. Wellburn, A. R. (1976) *Biochem. Physiol. Pflanz.* **169**, 265.
33. Whittle, K. J., Dunphy, P. J. and Pennock, J. F. (1966) *Biochem. J.* **100**, 138.
34. Meijboom, P. W. and Jongenotter, G. A. (1979) *J. Am. Oil. Chem. Soc.* **56**, 33.
35. Kato, T., Kumanireng, A. S., Ichinose, I., Kitahara, Y., Kakinuma, Y. and Kato, Y. (1975) *Chem. Letters*. 335.
36. Cillard, J., Moign, D., Cormmier, M. and Girre, R. L. (1975) *Plant Med. Phytother*, **9**, 224.
37. Soher, A., Vonov, I. S. A. and Seifen, F. (1973) *Anstrichimica* **75**, 606.
38. Karrer, P. and Solmssen, U. (1937) *Helv. Chim. Acta* **20**, 1396.