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FORMATION OF NATURAL THIOPHENE DERIVATIVES FROM ACETYLENES BY *TAGETES PATULA**

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Key Word Index—*Tagetes patula*; Compositae; biosynthesis; terthiophene; bithiophenylbutynol.

Abstract—Six tritium-labelled thiophene derivatives were synthesized and their emulsions were applied to whole plants of *Tagetes patula*. From the root extracts, naturally occurring 2,2′:5′,2″-terthiophene and 4-[2,2′-bithiophen-5-yl]-but-3-yn-1-ol were isolated and investigated for radioactivity. The four bithiophenyl precursors, as well as 1,1′-bis-[2-ethynyl-thiophene] and its methyl derivative, were incorporated into terthiophene, but only the bithiophenyl precursors were converted into bithiophenylbutynol. Whilst 5-(buta-1,3-diynyl)-2,2′-bithiophene and its 5′-methyl derivative are most probably the direct precursors of terthiophene, the 5-(but-3-en-1-ynyl)-2,2′-bithiophene and its 5′-methyl derivative are the precursors of the bithiophenyl-butynol. Incorporation of 1,1′-bis-[2-ethynylthiophene] and 2-[4-(2-thienyl)-buta-1,3-diynyl]-5-methyl-thiophene into terthiophene showed that the enzyme responsible for the thiophene ring formation has low specificity. From the feeding experiments with the methyl group substituted precursors, 5-methyl-terthiophene was trapped as an intermediate. The presence of the methyl group in the three types of precursors is obviously not important for further biosynthetic pathways.

INTRODUCTION

From previous feeding experiments it has been shown that the *in vivo* formation of thiophene rings takes place by formal addition of H_2S to diynes [1–4]. Incorporation of ^{35}S from $Na_2{}^{35}SO_4$ and [^{35}S]cysteine has been demonstrated [2, 5]. Results of feeding experiments with radioactively labelled pentayn-ene **2** (Scheme 1) allowed the ordering of these compounds into a hypothetical biogenetic scheme [1, 6]. The conversions from **2** to **9** and from **9** to 4-[2,2′-bithiophen-5-yl]-but-3-yn-1-ol (**12b**) were especially high, but of special interest was the transformation from **9** into 2,2′:5′,2″-terthiophene (**14**). Two biosynthetic pathways are possible. The authors could show by feeding of [1,2-$^3H_2$]-**2**, that the tritium atoms were located in the α-position of **14** only (1,12-position). Therefore it was concluded that **13** is not an intermediate in the biosynthesis of **14** and that the vinyl group in **9** is first dehydrogenated to an acetylenic group. As shown by feeding, **10** is an excellent precursor of **14**, but it is also converted into **9** [5] and further, in agreement with earlier result [1], into **12** [5]. These results demonstrate the ability of plants to hydrogenate triple bonds to double bonds and to form by addition of water or acetic acid saturated carbon chains.

The feeding results with [^{35}S]-**9** are not in agreement with the foregoing as high incorporation of **9** into **12** but none into **14** was demonstrated [7]. Therefore the methyl group (C-13) was supposed to be important in the further metabolic reactions and methylterthiophene (**11**) was supposed to be an intermediate [3, 4]. Furthermore, the sequence of thiophene ring formation was of interest. Preliminary feeding experiments on *Tagetes patula* were carried out with ^3H-labelled **5** and **7** [8]. To get a clearer picture, six ^3H-labelled possible precursors were synthesized and used in parallel feeding experiments.

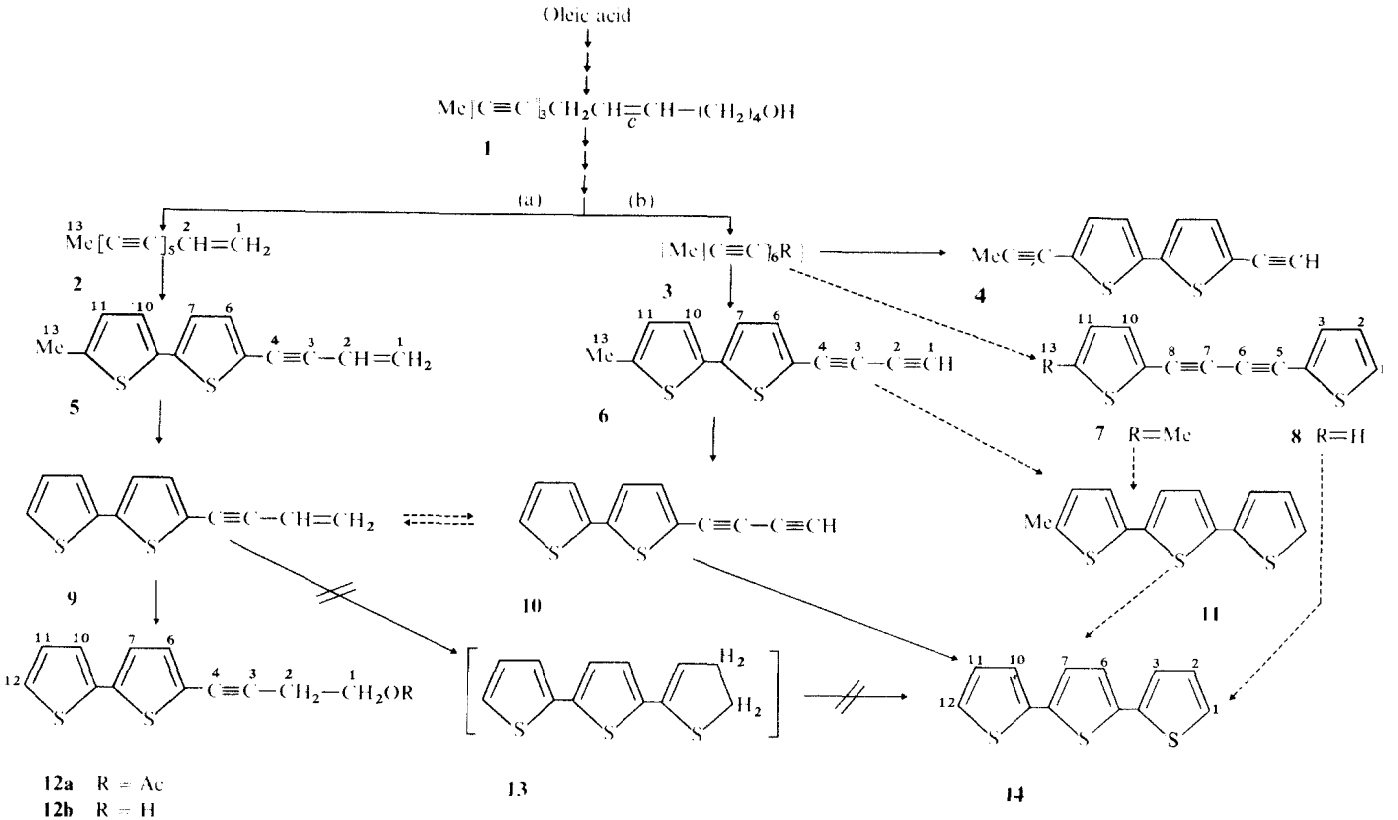
RESULTS AND DISCUSSION

Syntheses of the labelled compounds **5**–**10** started with ^3H-labelled thiophene ([3-^3H]-**16**) (Scheme 2).

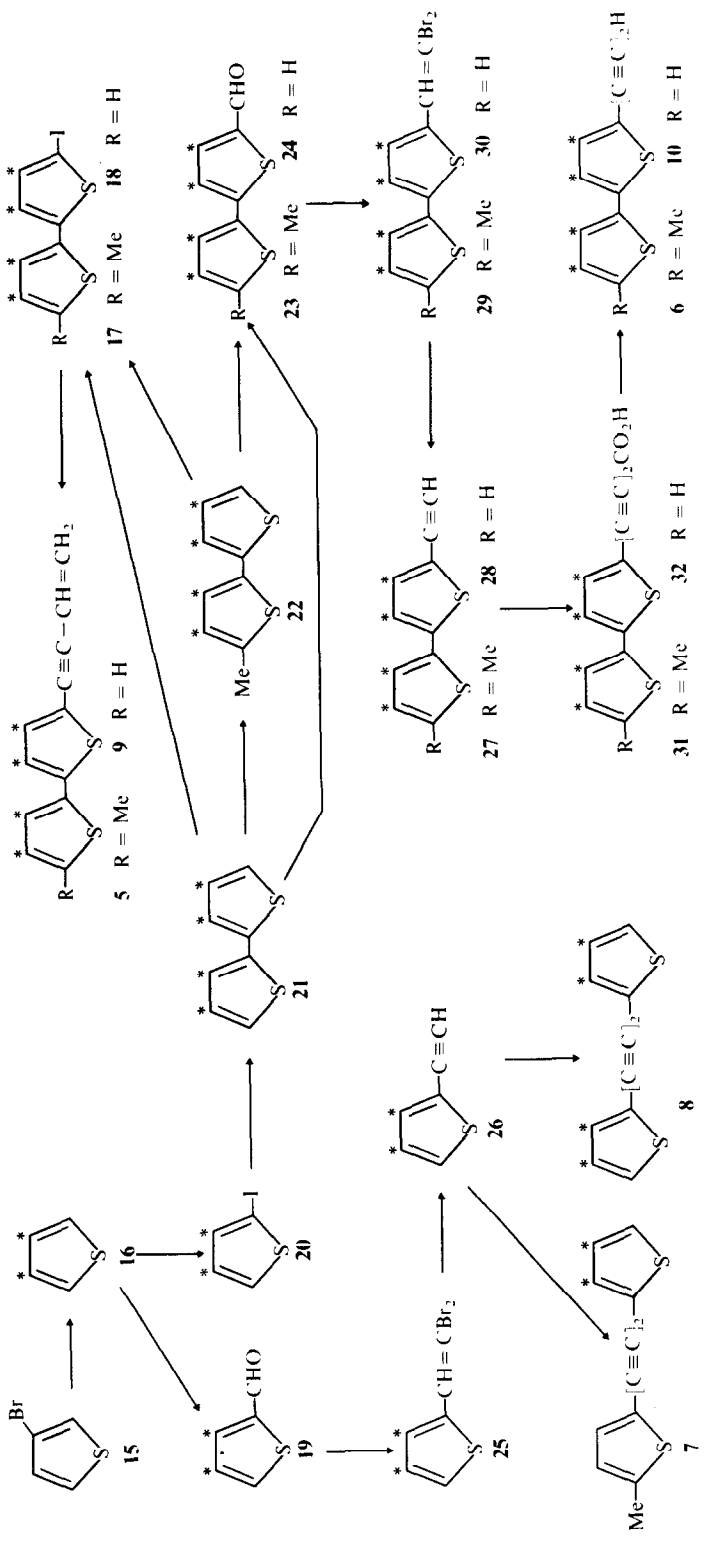
3-Bromo-thiophene (**15**) [9] was treated with butyl-lithium. The resulting 3-lithio derivative, on hydrolysis with tritiated water afforded labelled thiophene ([3-^3H]-**16**), which was converted to **20** [10]. Special conditions of the Ullmann reaction [11–13] afforded labelled bithiophene (**21**) in satisfactory yield. The Vilsmeier reaction and subsequent Wolff–Kishner reduction gave 5-methyl-2,2′-bithiophene (**22**) [13–16]. Compounds **21** and **22** were converted to the corresponding iodo derivatives **17** and **18** [11–13], which were coupled with copper butenyne [17] to yield the precursors **5** and **9**. Compounds **21** and **22** were transformed by the Vilsmeier reaction into the labelled aldehydes **23** and **24** [14, 15], with Ph_3P, Zn and CBr_4 into the dibromo compounds **29** and **30** and subsequently with butyl lithium into the acetylenes **27** and **28** [18]. Cadiot–Chodkiewicz coupling with bromopropiolic acid gave labelled **31** and **32** [19], which after decarboxylation afforded the precursors 5′-methyl-5-(buta-1,3-diynyl)-2,2′-bithiophene (**6**) and 5-(buta-1,3-diynyl)-2,2′-bithiophene (**10**) [20].

The tritiation method used unavoidably led to a thiophene solution in ether. To minimize loss of [3-^3H]thiophene during evaporation of the ether, *N*-methyl-formanilide, which also was a reagent in the next reaction, was added before distillation. The fraction with boiling point higher than 34° was redistilled after addition of some more *N*-methyl-formanilide. The residues of both distillations containing labelled thiophene were combined for the reaction leading to **19**. Compound **26** was prepared

* Dedicated to Prof. Dr. F. Bohlmann on his 60th birthday.



Scheme 1. Biosynthetic pathways to thiophenes in *Tagetes patula*.



Scheme 2. Syntheses of the labelled precursors. (* position of tritium label. As **16** is symmetrical the tritium label is in both β-positions.)

in analogy to the preparation of **27** and **28**. Glaser coupling [21, 22] of **26** with 2-ethynyl-5-methylthiophene and with itself afforded the precursors **7** and **8**.

The emulsions of the labelled compounds **5**–**10** were applied under the same conditions to whole plants of *Tagetes patula*. Compounds **14** and **12a** were isolated from the root extract by column chromatography and TLC. The oily acetate **12a** was hydrolysed to the corresponding alcohol **12b**. Feeding of compounds **5**–**7**, which still have the methyl substituent, should show if compound **11**, not yet found in this plant, is also formed in addition to **14**. However, since only very small amounts of labelled **11** could be expected, none labelled **11** was added to the corresponding fractions as carrier. Compounds **14**, **12b** and in three experiments also **11** were crystallized to constant specific radioactivity. The results are given in Table 1 and Fig. 1.

All compounds were incorporated into terthiophene (**14**). Incorporations into **14** were best, and in nearly equal yields, with the diynes **6** and **10**, but low incorporations were also found with the butenyne precursors **5** and **9** (0.1 and 0.4% of the incorporations of **6** and **10**). The diynes **6** and **10** are therefore most probably direct precursors for **14** which is in agreement with the scheme proposed by Bohlmann [6]. Schulte *et al.* [5, 7] found high incorporation of **10** into **14**, but the conversion of **9** to **14** was not observed by these authors. This may be due to a much lower specific activity of **9** and use of a much smaller amount of plant material and therefore the radioactivity of **14** was perhaps too low for detection.

As already shown by Bohlmann *et al.* [1] as well as by Schulte *et al.* [5, 7], **9** is a good precursor for the bithiophene derivative **12**. Incorporation of the methyl derivative **5** was less when compared with the incorporation of **9** into **12**, probably due to the fact that **9** occurs in the root of this plant as a natural compound. Also, in agreement with the results of Schulte [5], there was a low incorporation of **10** into **12** (0.4% and 1.5% of the incorporations of **9** and **5**, respectively) and the same was true for **6**. Both conversions involve a hydrogenating step from a diyne grouping to an enyne grouping, which seems to be somewhat faster than the reverse dehydrogenation step. Compounds **7** and **8** were also converted to **14** in remarkable yields. This demonstrates that the sequence of thiophene ring formation is not important in the plant cell metabolism. From the feeding experiments with the labelled precursors **5**–**7** biosynthetically formed **11** was indeed identified by its radioactivity. Probably the enzyme forming the thiophene ring is not of high specificity and can utilize the different conjugated diyne groups.

The results of the feeding experiments with the three pairs of methyl and demethyl precursors (**5** and **9**, **6** and **10**, **7** and **8**) demonstrate that the methyl group does not cause basic changes of the following metabolic conversions. Only the conversion rate to the isolated products may be altered. The feeding experiments with the labelled derivatives **5**–**7**, on the one hand, and **8**–**10** on the other, showed that the three thiophene rings of the terthiophene **14** can be produced from both types of precursors. As the incorporation rates of **6** and **10** into **14** and **6** and **10** into **12** respectively, were comparable, the conversion of **6** to **10** is very fast, in relation to the subsequent reactions and therefore this conversion is important in the methyl group elimination in the biosynthetic pathway to **14**. The elimination is probably

Table 1. Results of the feeding experiments

Precursor	Isolated compound			
Total activity (dpm)	Compound	Total amount (mmol)	Total activity (dpm)	Incorporation (%)
[6,7,10,11-$^3H_4$]-**5**	**14**	0.048	5.52×10^3	0.001
(5.4×10^8)	**12a**	0.060	7.14×10^5*	0.132
	11	≥0.076†	$\geq 3.19 \times 10^4$	≥0.006
[6,7,10,11-$^3H_4$]-**9**	**14**	0.097	4.8×10^4	0.005
(9.3×10^8)	**12a**	0.077	4.4×10^6*	0.470
[6,7,10,11-$^3H_4$]-**6**	**14**	0.133	3.28×10^6	1.250
(2.6×10^8)	**12a**	0.051	6.23×10^3*	0.002
	11	≥0.076†	$\geq 3.08 \times 10^4$	≥0.012
[6,7,10,11-$^3H_4$]-**10**	**14**	0.077	4.03×10^6	1.160
(3.5×10^8)	**12a**	0.038	7.52×10^3*	0.002
[2,3-$^3H_2$]-**7**	**14**	0.085	9.27×10^4	0.066
(1.4×10^8)	**12a**	0.038	0*	0
	11	≥0.076†	$\geq 1.55 \times 10^4$	≥0.011
[2,3,10,11-$^3H_4$]-**8**	**14**	0.121	1.26×10^6	0.385
(3.3×10^8)	**12a**	0.051	0*	0

* Determined after hydrolysis to **12b**.

† Unlabelled compound (0.076 mmol) was added as carrier.

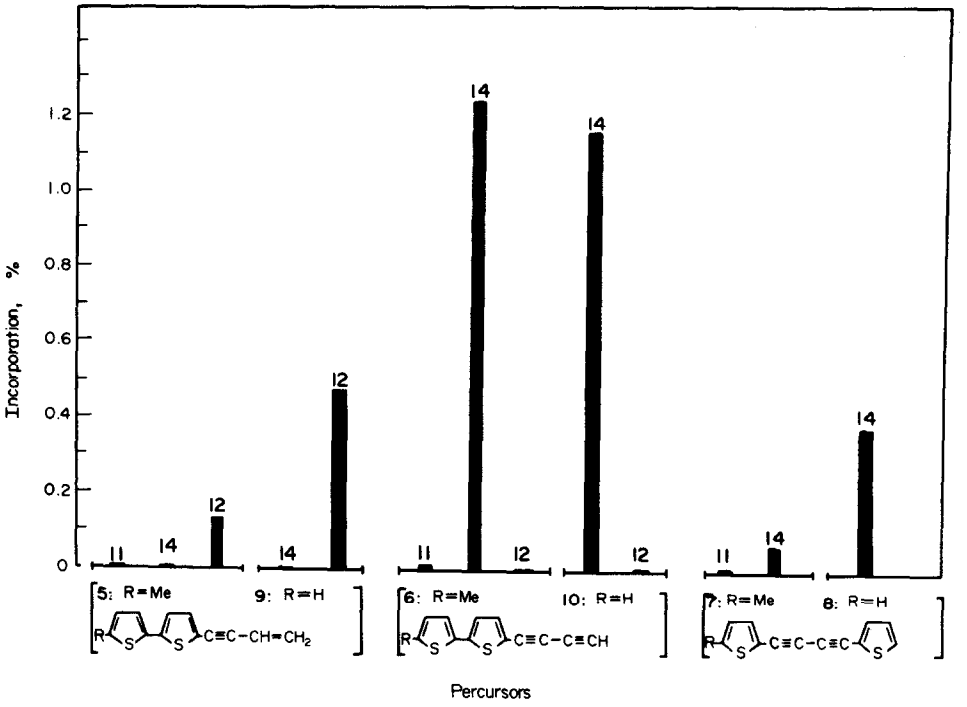


Fig. 1. Incorporation of the precursors.

an oxidative decarboxylation process [3,4]. In the presence of diyne groupings this decarboxylation should be favoured.

Summarizing our present knowledge, it can be proposed that **2**, **5** and **9** as well as **7** and **8**, though converted to **14**, are not necessarily 'true' intermediates in the biosynthetic pathway to **14**. Considering the yields found for the conversions, Scheme 1 is most likely.

Probably in the route from oleic acid to the naturally occurring thiophene derivatives of *Tagetes patula*, the pathway splits into two routes. While the first route (line a) leads to the bithiophenylbutynol **12**, the other route (line b), in which one more triple bond becomes introduced into the intermediate, leads to the terthiophene **14**. The possibility of **11** being an intermediate in the pathway to **14** cannot be excluded. The importance of **6** and **10**, so far not detected in nature, is supported by the isolation of **4** from the flowers of *Tagetes erecta* [23].

EXPERIMENTAL

General methods. ^{1}H NMR: 270 MHz $CDCl_3$, TMS as internal standard. Radioactivity: in toluene with dimethyl-POPOP and PPO, quench corrections using [^{3}H]toluene as internal standard. Column chromatography (CC): Al_2O_3 (acidic, grade II) or Si gel eluted with petrol with increasing quantities of Et_2O; Sephadex LH-20 (MeOH–$CHCl_3$; 1:1). Prep. TLC (0.3 mm): Si gel PF 254/petrol–Et_2O. Distillation temps were those of the air bath.

Organic layers of extractions were generally washed with H_2O until neutral, then dried with $MgSO_4$, filtered and the solvents evapd under red. pres. In some cases unlabelled material was added to the labelled compounds as carrier for complete isolation or purification.

The physical data of the compounds synthesized were identical with those in the literature unless especially mentioned.

The natural compounds were identical with authentic material.

Syntheses of the labelled precursors

[^{3}H]*Thiophene* [^{3}H]-**16**. 3-Bromothiophene (5.78 g) in 15 ml dry Et_2O was added dropwise to a soln of 39.3 mmol BuLi in 10 ml hexane at −78° and after 30 min 400 μl ^3H_2O (*ca* 0.8 Ci) were added. The mixture was warmed up and 400 μl H_2O were added slowly. After 30 min more H_2O, 3 ml of unlabelled thiophene and 2 ml C_6H_6 were added. The mixture was shaken and the organic phase was sepd. The aq. phase was shaken with more C_6H_6 and the C_6H_6 layers were combined.

For the syntheses of the labelled compounds **5**, **6**, **9** and **10** one part of the C_6H_6–thiophene mixture was distilled using a column. First, a fraction with bp up to 40° was collected, followed by a second fraction with bp up to 78°. To the second fraction unlabelled thiophene and more C_6H_6 were added and this fraction was redistilled up to 78°. The residues of these two distillations consisting of C_6H_6 and thiophene were combined and distilled as a C_6H_6–thiophene mixture.

Labelled 5-(*but*-3-*en*-1-*ynyl*)-5′-*methyl*-2,2′-*bithiophene* (**5**) *and* 5-(*but*-3-*en*-1-*ynyl*)-2,2′-*bithiophene* (**9**). To the labelled C_6H_6-thiophene mixture (8 g [^{3}H]-**16**, estimated) HgO (17 g) and iodine (24.8 g) were added alternately in small portions with vigorous shaking in an ice bath. At the end of the reaction the colour of the ppt. became crimson (HgI_2). The ppt. was filtered and washed with Et_2O. The filtrate was washed with an aq. soln of $Na_2S_2O_3$ in order to remove dissolved iodine. The organic layer was evapd and the product distilled, bp 116°/45 torr, yield: 11 g (55%) [$^3H_2$]-**20**. The Ullmann reaction was carried out with [^{3}H]-**20** (11 g) in DMF (30 ml) and copper powder (3.94 g). The mixture was refluxed with stirring for 2 hr. After cooling, H_2O was added and the product was extrd with Et_2O and distilled, bp 115°/0.01 torr, yield: 2.6 g (59%) [$^3H_4$]-**21**. In the Vilsmeier

reaction (*a*) [$^3H_4$]-**22** (0.329 g), (*b*) [$^3H_4$]-**21** (1.7 g) were stirred with $POCl_3$ [(*a*) 0.31 g, (*b*) 1.7 g] and *N*-methyl-formanilide [(*a*) 0.273 g, (*b*) 1.47 g] for 20 min at 100°.

After cooling a satd aq. soln of NaOAc and Et_2O were added. After stirring for 30 min the Et_2O layers were removed and aq. phases extrd with Et_2O. The organic layers were combined, washed with dil. HCl, aq. $NaHCO_3$ and H_2O. The products were distilled, bp 120°/0.01 torr, yields: (*a*) 273 mg (72%) [$^3H_4$]-**23**, (*b*) 1.5 g (76%) [$^3H_4$]-**24**.

For Wolff–Kishner reduction [$^3H_4$]-**24** (800 mg) in 4.5 ml 1,2-ethanediol was heated to 100° (bath temp.), 0.89 ml 80% hydrazine hydrate were added and the mixture was heated at 190° for 10 min, after allowing the temp. to fall to 100°, 0.89 g of powdered KOH were added and the temp. was raised again to 190° for 20 min. After cooling, H_2O was added and the product extracted with Et_2O. The product was distilled, bp 130°/0.02 torr, yield: 465 mg (63%) [$^3H_4$]-**22**.

(*a*) [$^3H_4$]-**22** (138 mg) as well as (*b*) [$^3H_4$]-**21** (300 mg) were treated with HgO and iodine as described for the synthesis of **20**. Yields: (*a*) 233 mg (99%) [$^3H_4$]-**17**, (*b*) 500 mg (95%) [$^3H_4$]-**18**. (*a*) [$^3H_4$]-**17** (233 mg) in 5 ml pyridine and (*b*) [$^3H_4$]-**18** (378 mg) in 10 ml pyridine were refluxed with (*a*) 118 mg, (*b*) 198 mg copperbutenyne for 3 hr. The mixtures were poured on ice and extrd with Et_2O. The products were purified by prep. TLC and CC (Sephadex LH-20). Yields: (*a*) 69 mg (40%) [6,7,10,11-$^3H_4$]-**5**, UV $\lambda_{max}^{Et_2O}$ nm: 350, 250 nm [24], sp. act.: 6.2×10^9 dpm/mmol; (*b*) 84 mg (30%) [6,7,10,11-$^3H_4$]-**9**, UV $\lambda_{max}^{Et_2O}$ nm: 343, 248 [25], sp. act.: 1.0×10^{10} dpm/mmol.

Labelled 5-(buta-1,3-diynyl)-5'-methyl-2,2'-bithiophene (**6**) *and 5-(buta-1,3-diynyl)-2,2'-bithiophene* (**10**). In round-bottom flasks were placed Ph_3P (*a*: 0.95 g; *b*: 1.36 g), zinc (*a*: 0.24 g; *b*: 0.35 g), CBr_4 (*a*: 1.19 g; *b*: 1.7 g) and 5 ml of dry CH_2Cl_2. The flasks were immediately sealed with serum caps and the mixtures were stirred at 20° for 28 hr. Then 400 mg [$^3H_4$]-**23** were added to *a* (see syntheses above), or 400 mg [$^3H_4$]-**24** were added to *b*; each was dissolved in 2 ml CH_2Cl_2 and they were added dropwise from a syringe with stirring. After 2 hr, petrol was added, the mixtures were filtered, the filtrates evapd and the residues purified by prep. TLC (petrol). Yields: (*a*) 480 mg (69%) [$^3H_4$]-**29** and (*b*) 700 mg (97%) [$^3H_4$]-**30**. A soln of 20% BuLi in hexane (*a*: 1.53 ml; *b*: 2.0 ml) was added at −78° with a syringe to (*a*) [$^3H_4$]-**29** (480 mg), (*b*) [$^3H_4$]-**30** (700 mg) in 10 ml of dry Et_2O–THF (1:1). The mixtures were stirred for 1 hr at −78° and for 1 hr at 20°, then H_2O was added and the mixtures were stirred for another 30 min. The products were extrd with Et_2O and purified by fast CC (Si gel). Yields: (*a*) 265 mg (98%) [$^3H_4$]-**27**, (*b*) 370 mg (97%) [$^3H_4$]-**28**. (*a*) [$^3H_4$]-**27** (265 mg) as well as (*b*) [$^3H_4$]-**28** (370 mg) were dissolved each in 4 ml MeOH–THF (1:1) and Cu_2Cl_2 (*a*: 3.2 mg, *b*: 4 mg), hydroxylamine hydrochloride (*a*: 41 mg, *b*: 50 mg), 40% ethylamine (*a*: 0.36 ml *b*: 0.44 ml) and finally propiolic acid (*a*: 243 mg, with 0.4 ml 40% ethylamine neutralized, *b*: 300 mg, with 0.48 ml 40% ethylamine) in 4 ml MeOH were added. It was stirred for 15 min at 0°, 1 hr at 20°; the mixtures were shaken with Et_2O and the organic layers discarded. Then the solns were acidified with HCl and the two acids [$^3H_4$]-**31** and [$^3H_4$]-**32** extracted with Et_2O. Yields: (*a*) 134 mg (38%) [$^3H_4$]-**31**, (*b*) 186 mg (37%) [$^3H_4$]-**32**.

The labelled compounds **31** and **32** were each dissolved in 20 ml dry THF, 1 g tetramine copper II sulfate was added and the mixtures were refluxed for 15 min. After the addition of H_2O the products were extracted with Et_2O and purified by prep. TLC (petrol) and CC (Sephadex LH-20). Yields (*a*) 55 mg (49%) [6,7,10,11-$^3H_4$]-**6**; UV $\lambda_{max}^{Et_2O}$ nm: 350, 256; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3310; 2210 (C≡C–H); sp. act.: 3.0×10^9 dpm/mmol. (*b*) 63 mg (40%) [6,7,10,11-$^3H_4$]-**10**; UV $\lambda_{max}^{Et_2O}$ nm: 346, 250; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3310, 2210 (C≡C–H); sp. act.: 3.67×10^9 dpm/mmol.

Labelled 2-[4-(2-thienyl)-buta-1,3-diynyl]-5-methylthiophene (**7**) *and 1,1'-bis-[2-ethynyl-thiophene]* (**8**). To the remaining labelled thiophene ([^3H]-**16**) in C_6H_6 (see above) 2 ml of *N*-methyl-formanilide were added, then Et_2O and a further fraction (up to 78°) were removed by distillation. The latter fraction after addition of some more unlabelled thiophene and some more *N*-methylformanilide were redistilled and the residues of the two distillations were combined. The labelled thiophene was converted into [$^3H_2$]-**19** as previously described for the reaction with **21**, bp: 110°/*ca* 40 torr, yield: 2.15 g [$^3H_2$]-**19**.

[$^3H_2$]-**19** (800 mg) was converted to [$^3H_2$]-**25** with Ph_3P (4.8 g), zinc (1.19 g) and CBr_4 (6.0 g) in 15 ml CH_2Cl_2 using the same procedure described previously for syntheses of [$^3H_4$]-**29** and [$^3H_4$]-**30**. [$^3H_2$]-**25** (1.22 g) was then converted to [$^3H_2$]-**26** by treatment with 14 mmol BuLi in the same manner as described for the syntheses of labelled **27** and **28**. Yield: 490 mg (99%) [$^3H_2$]-**26**.

To (*a*) [$^3H_2$]-**26** (100 mg) and unlabelled 2-ethynyl-5-methyl-thiophene (300 mg) in 4 ml MeOH, (*b*) [$^3H_2$]-**26** (124 mg) in 4 ml MeOH were added (*a*) 6.25 ml, (*b*) 1.75 ml of the aq. soln made from 0.8 g Cu_2Cl_2, 2.4 g NH_4Cl and 10 ml H_2O, acidified with HCl to pH 5.0. The mixtures were shaken vigorously for 2 hr under an atmosphere of O_2. Then the products were transferred in Et_2O and purified by CC (Sephadex LH-20). Yields: (*a*) 32 mg (15%) [2,3-$^3H_2$]-**7**; UV $\lambda_{max}^{Et_2O}$ nm: 359; 337; 317; sp. act.: 1.58×10^9 dpm/mmol. (*b*) 56 mg (46%) [2,3,10,11-$^3H_4$]-**8**; mp 91° [26]; UV $\lambda_{max}^{Et_2O}$ nm: 352; 332; 313 [27]; sp. act.: 3.5×10^9 dpm/mmol.

Feeding experiments

For each expt 20 mg of the labelled precursors **5**–**10** were combined with 15 mg sunflower seed oil and 90 ml 0.001% saccharose palmitate in tap-water and emulsified using the 'Ultra-Turrax'. Into these emulsions the plants were placed after their roots were washed. Tap-water was added after uptake of the emulsion. After 40 hr feeding time the roots (about 275 g per expt) were chopped and extracted twice with Et_2O–petrol (1:1) and then once with Me_2CO–MeOH (1:1). The Me_2CO–MeOH extracts were evapd and the residues shaken with Et_2O–petrol. The organic layers were combined with the Et_2O–petrol extracts, dried, filtered and the solvent evapd. The compounds were sepd by CC (Al_2O_3/petrol–Et_2O). Compound **14** was eluated with petrol and **12a** with petrol–Et_2O (20:3). The two compounds were further purified by prep. TLC (petrol–Et_2O, 10:1) and CC (Sephadex LH-20). The CC was repeated after the addition of unlabelled carrier (5 mg) of the same compound fed.

Saponification of **12a**. 2 ml of an alkaline reagent (5 g KOH in 100 ml of MeOH–H_2O, 7:3) were added to the compound and the mixture shaken in a water-bath (55°) for 2 min. Immediately ice was added and **12b** extracted with Et_2O. Compound **12b** was purified by prep. TLC (petrol–Et_2O, 5:1). With alternate filtrations over charcoal, **14** and **12b** were recryst. from petrol until constant sp. act. was achieved.

To the appropriate fractions of the Sephadex CC preceding **14** from the extracts of the feeding expts with **5**–**7** 20 mg of unlabelled **11** (synthesized from **14** by the method described for **22**) were added and chromatographed (Sephadex LH-20) again for separation of **11**, which was recryst. to constant sp. act. The experimental data and the results of the feeding expts are given in Tables 1 and 2.

Acknowledgements—We thank Prof. Dr. F. Bohlmann for kind discussion and the Deutsche Forschungsgemeinschaft for financial support.

Table 2. ^{1}H NMR spectral data of the precursors **5–10**

H	5	9	6	10	7	8
1	5.64 *dd* 5.46 *dd*	5.74 *dd* 5.57 *dd*	2.67 *s*	2.68 *s*	7.31 *dd*	7.34 *dd*
2	5.95 *dd*	6.04 *dd*	—	—	6.99 *dd*	7.01 *dd*
3	—	—	—	—	7.32 *d*	7.36 *dd*
6	6.99 *d*	7.10 *d*	7.23 *d*	7.26 *d*	—	—
7	6.86 *d*	7.04 *d*	6.93 *d*	7.03 *d*	—	—
10	6.89 *d*	7.18 *dd*	7.00 *d*	7.21 *dd*	7.15 *d*	7.36 *dd*
11	6.59 *dq*	7.03 *dd*	6.68 *dq*	7.03 *dd*	6.66 *d*	7.01 *dd*
12	—	7.23 *dd*	—	7.27 *dd*	—	7.34 *dd*
13	2.40 *d*	—	2.49 *d*	—	2.49 *s(br)*	—

J(Hz): **5, 9**: 1,1′ = 2; 1,2 = 17.5; 1′,2 = 11; **5, 6, 9, 10**: 6,7 = 10,11 = 3.8; **9,10**: 10,12 = 1; 11,12 = 5; **5, 6**: 11,13 = 1; **7,8**: 1,2 = 5; 1,3 = 1.2; 2,3 = 10,11 = 3.5; **8**: 10,12 = 1.2; 11,12 = 5.

REFERENCES

1. Bohlmann, F. and Hinz, U. (1965) *Chem. Ber.* **98**, 876.
2. Schulte, K. E., Rücker, G. and Meinders, W. (1965) *Tetrahedron Letters* 659.
3. Bohlmann, F. (1967) *Fortschr. Chem. Org. Naturst.* **25**, 1.
4. Bohlmann, F ¯ rkhardt, T. and Zdero, C. (1973) *Naturally Occurring Acetylenes*. Academic Press, New York.
5. Schulte, K. E., Henke, G., Rücker, G. and Foerster, S. (1968) *Tetrahedron* **24**, 1899.
6. Bohlmann, F., Wotschokowsky, M., Hinz, U. and Lucas, W. (1966) *Chem. Ber.* **99**, 984.
7. Schulte, K. E. and Foerster, S. (1966) *Tetrahedron Letters* 773.
8. Brunke, R. (1977) Dissertation Technical University, Berlin.
9. Reinecke, M. G., Adickes, H. W. and Pyun, C. (1971) *J. Org. Chem.* **36**, 2690.
10. Minnis, W. (1943) *Org. Syntheses*, Coll. Vol. 2, p. 357.
11. Steinkopf, W., v. Petersdorff, H. J. and Gording, R. (1937) *Ann.* 527, 272.
12. Steinkopf, W., Leitsmann, R. and Hofmann, K. H. (1941) *Ann.* **546**, 180.
13. Uhlenbroek, J. H. and Bijloo, J. D. (1960) *Rec. Trav. Chim.* **79**, 1181.
14. Vilsmeier, A. (1951) *Chem.-Ztg* **75**, 133.
15. Houben-Weyl (1954) *Methoden d. Org. Chemie*. Vol. VII, part 1, p. 29.
16. King, W. J. and Nord, F. F. (1949) *J. Org. Chem.* **14**, 638.
17. Atkinson, R. E., Curtis, R. F. and Phillips, G. (1964) *Chem. Ind.* 2101.
18. Corey, E. J. and Fuchs, P. L. (1972) *Tetrahedron Letters* 3769.
19. Chodkiewicz, W., Alhuwalia, J. S., Cadiot P. and Willemart, A. (1957) *C. R. Acad. Sci.* **245**, 322.
20. Bohlmann, F., Sucrow, W. and Queck, I. (1964) *Chem. Ber.* **97**, 2586.
21. Glaser, C. (1870) *Ann.* **154**, 137.
22. Eglinton, G. and McCrae, W. (1963) *Adv. Org. Chem.* **4**, 225.
23. Bohlmann, F., Grenz, M., Wotschokowsky, M. and Berger, E. (1967) *Chem. Ber.* **100**, 2518.
24. Bohlmann, F. and Berger, E. (1965) *Chem. Ber.* **98**, 883.
25. Uhlenbroek, J. H. and Bijloo, J. D. (1959) *Rec. Trav. Chim.* **78**, 382.
26. Schulte, K. E., Reisch, J. and Hörner, L. (1962) *Chem. Ber.* **95**, 1943.
27. Vaitiekunas, A. and Nord, F. F. (1954) *J. Am. Chem. Soc.* **76**, 2733.