

## Tumor Inhibitors. 3. Identification and Synthesis of an Oncolytic Hydrocarbon from American Coneflower Roots<sup>1</sup>

Denys J. Voaden and Martin Jacobson\*

Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland 20705.  
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A constituent of the root oil of *Echinacea angustifolia* DC. and *E. pallida* (Nutt.) Britt. inhibitory to Walker carcinosarcoma 256 and P-388 lymphocytic leukemia was isolated and identified as (Z)-1,8-pentadecadiene (2). This compound occurs in these oils to the extent of approximately 44% and appears to be the first diene olefin reported to show *in vivo* antitumor activity. The corresponding trans isomer (16) is less active. Both isomers were synthesized.

The genus *Echinacea* (synonyms: *Brauneria*, *Rudbeckia*), family Compositae, contains 3<sup>2</sup> or 4<sup>3,4</sup> species of flowering herbaceous perennials with brightly colored heads. It is native to the central or west-central United States,<sup>5</sup> ranges from Texas and Alabama to Saskatchewan in Canada,<sup>3,6</sup> and is known as the "American coneflower."

*Echinacea angustifolia* DC. was known by at least 6 names to various Indian tribes of Nebraska among whom it had a reputation for antiseptic and analgetic properties.<sup>7</sup> From 1887, the plant was incorporated into certain patent medicines—of disputed worth since a medical committee later roundly disavowed its therapeutic value.<sup>8</sup> However, the dried rhizome and roots of *E. angustifolia* and *E. pallida* (Nutt.) Britt., and the alcoholic extracts thereof, were for a time adopted into the armamentarium of the modern pharmacist.<sup>9</sup> Thus the plant probably had no more than a tonic value, but the attribution of diaphoretic and healing properties has persisted.<sup>10</sup>

More recent investigations have shown that extracts of *E. purpurea* Moench such as the commercial echinacin have an antiexudative property which can bring about some healing in wounds or bruises.<sup>11</sup> *E. purpurea* has been cultivated in Europe for homeopathic medicine, and an analysis of the oil content of various parts of the plant has been reported.<sup>12</sup>

Among the known constituents of *Echinacea* species are echinacoside, a caffeic acid glycoside,<sup>13</sup> echinacein [(*E,Z,E,E*)-*N*-isobutyl-2,6,8,10-dodecatetraenamide], a sialagog which is very toxic to house flies, *Musca domestica* L., and is identical with neoherculin and  $\alpha$ -sanshool;<sup>14</sup> at least 4 other closely related isobutylamides;<sup>15</sup> and more than a dozen polyacetylenes.<sup>16</sup> Also, the roots of *E. angustifolia* contain about 1 part in 2500 of 8-pentadecen-2-one<sup>16</sup> and as yet unidentified substances capable of disrupting insect development.<sup>17</sup>

Incidental to the isolation of the insecticidal principle echinacein,<sup>14</sup> and in continuation of the search for tumor inhibitors from plant and insect sources, a pentane extract of the roots of *E. pallida* was screened for oncolytic activity. It showed inhibitory activity, especially in Walker carcinosarcoma 256 (WA); in addition, the distilled, pentane-soluble oil from roots of *E. angustifolia* subsequently showed confirmed activity against both WA and P-388 lymphocytic leukemia (PS).† The isolation and characterization of the oncolytic principle is here described.

**Chemistry.** The roots of *E. angustifolia* contain a volatile oil with a characteristic odor; the fraction obtained by

EtOH extraction was examined by Bischoff<sup>19</sup> and Woods.<sup>20</sup> The former showed that the principal component was a C<sub>15</sub> diene and considered that it contained some branching. Woods carried out more extensive oxidation of this fraction with aqueous MnO<sub>4</sub><sup>-</sup> and obtained AcOH, heptanoic and/or hexanoic acid, a supposed dihydroxy C<sub>13</sub> acid, and adipic and pimelic acids. Also, after oxidation in acetone solution, he isolated the parent of the dihydroxy compound, thought to be a tridecanoic acid. If the hexanoic acid was isohexanoic (4-methylpentanoic) acid, Woods felt that the C<sub>15</sub>H<sub>28</sub> component of *Echinacea* root oil was a mixture of 2 isomeric dienes, namely 2-methyl-5,12-tetradecadiene and 2-methyl-6,12-tetradecadiene. He also isolated a small amount of myristic acid from the oxidation mixture and postulated a 5–10% content of 1-pentadecene in the oil to account for its presence.

The root oils of *E. angustifolia* and *E. pallida* used in the present investigation were fractionally distilled to obtain samples as homogeneous as possible. The only differences seen on glc examination were in the minute amounts of lower-boiling substances not removed with the foreruns. On glc columns, the oil from each species exhibited a large peak in the region expected for a C<sub>15</sub> hydrocarbon plus 10–15% of a closely related substance. The 2 components were separated by chromatography on a silicic acid-AgNO<sub>3</sub> column, and the minor component was identified as 1-pentadecene.

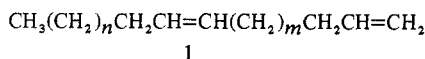
Elemental analysis of the oncolytic distilled hydrocarbon oil confirmed the C<sub>15</sub>H<sub>28</sub> formula of the earlier workers, and the ir spectrum showed that the substance was a long-chain olefin possessing a terminal double bond but lacking trans unsaturation or an R<sub>3</sub>CH structure. The nmr spectrum showed that a Me group, a CH<sub>2</sub> chain, and an allylic CH<sub>2</sub> were present. Also a complex multiplet was present in the olefinic region of the spectrum which was calcd from integration of the curve to comprise 5 H atoms. The remaining 23 hydrogens were in the ratio 3 (one Me):14 (–CH<sub>2</sub>–chain):6 (allylic). The structure of the hydrocarbon therefore appeared to be 1.

Hydrogenation with a PtO<sub>2</sub> or Pd catalyst gave a pentadecane identical in ir and glc characteristics with authentic *n*-pentadecane. R<sub>3</sub>CH was not evident in the ir spectrum, and it was concluded that the principal *Echinacea* diene is unbranched. This is not in accord with the opinions of Bischoff and Woods whose assignment of branching was based on the nonidentity of C<sub>6</sub> and C<sub>7</sub> acids obtained by oxidative degradation with hexanoic and heptanoic acids. It now seems likely that the oxidative acid fractions (which Woods admittedly did not claim to be pure single substances) were actually mixtures of these 2 acids (and perhaps lower homologs—cf. Experimental Section). Woods'

†The *in vivo* inhibitory activity was assayed under the auspices of Drug Research and Development, National Cancer Institute, National Institutes of Health, Public Health Service, by the procedure described in ref 18.

analysis for Ag in the salt from a fraction which he thought might be largely "isobutylic" acid is actually intermediate between those for silver hexanoate and silver heptanoate. Nevertheless, the methyltetradecadiene formulation postulated is sufficiently close to the pentadecadiene structure now presented to be regarded as an excellent effort from a period when ir, glc, and nmr were not available. The possibility that the earlier workers actually isolated a branched-chain hydrocarbon seems remote since the fraction used by Woods in his oxidation study was a middle cut from a representative portion of the root extract and had the same boiling point, 280–285° (760 mm), as the distilled oil used in the present investigation. The fractions seem to be the same material.

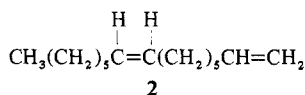
The position of the internal double bond was sought by oxidative degradation. Treatment of the diene with O<sub>3</sub> and then with a reducing agent gave CH<sub>2</sub>O and heptanal, indication that *n* = 4 and *m* = 4 in 1. Hence, the active



*Echinacea* hydrocarbon is (*Z*)-1,8-pentadecadiene (2). The ozonides were subjected to high-temp catalytic reduction (C-skeleton analysis),<sup>21</sup> which is accompanied by decarbonylation of aldehyde fragments. The products were hexane (from heptanal) and pentane (from pimelaldehyde).

The foregoing view of the diene structure was upheld by studying the ozonolysis in the presence of 2,4-dinitrophenylhydrazine when tlc permitted identification of the 2,4-dinitrophenylhydrazones of CH<sub>2</sub>O and heptanal. Pimelic dialdehyde and its corresponding dihydrazone were not sought during this investigation. The addition of authentic specimens to the reaction mixture showed that the hydrazone of CH<sub>2</sub>O, but not that of AcH, was present. The latter would be expected if Woods' structure were correct.

Further support for structure 2 was obtained by studying



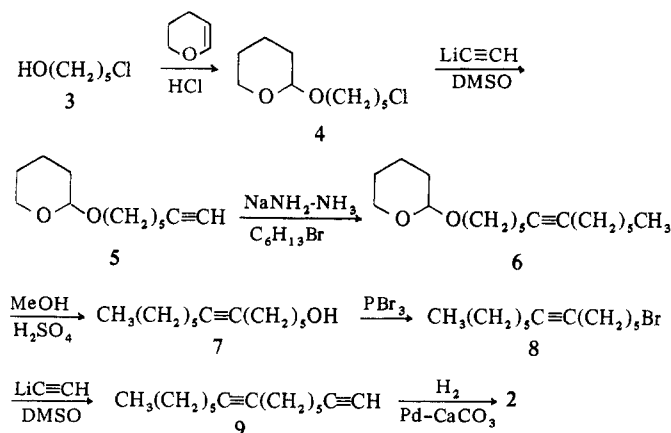
the acids produced by oxidation. Large-scale oxidation of distilled *Echinacea* oil with aqueous KMnO<sub>4</sub> cleaved both double bonds to give, besides myristic acid from the pentadecene, CO<sub>2</sub> from the terminal C, fatty acids, ranging from propionic to heptanoic, and adipic acid. Ideally, one should obtain heptanoic and pimelic acids and CO<sub>2</sub>. The lower fatty acids presumably arise from stepwise degradation of the heptanoic acid or from a primary intermediate produced by the permanganate attack. The susceptibility to oxidation is shown by the formation of propionic acid or possibly AcOH. Such a progressive chain shortening, rather than the presence of an ethylidene group in the molecule, could account for Woods' isolation of AcOH. Thus, supposed 11-methyl-7-dodecenoic acid<sup>20</sup> is more likely to have been 7-tetradecenoic acid; the reported neutralization equivalent and the results of the Ag salt analysis are in somewhat better agreement for this C<sub>14</sub> fragment than for the C<sub>13</sub> acid.

The diene, though unconjugated, is easily attacked by atmospheric O<sub>2</sub>, which gradually converts it to a gummy, rancid material. Deterioration of the oil<sup>19,20</sup> and of *Echinacea* roots and extracts<sup>9</sup> has been mentioned in the earlier literature.

To confirm that the antitumor activity was actually caused by 2, we prepared this compound synthetically. The most convenient routes were *via* the selective reduction of acetyl-

enes. A rational construction of the molecule, shown in Scheme I, commenced by converting 5-chloro-1-pentanol

Scheme I

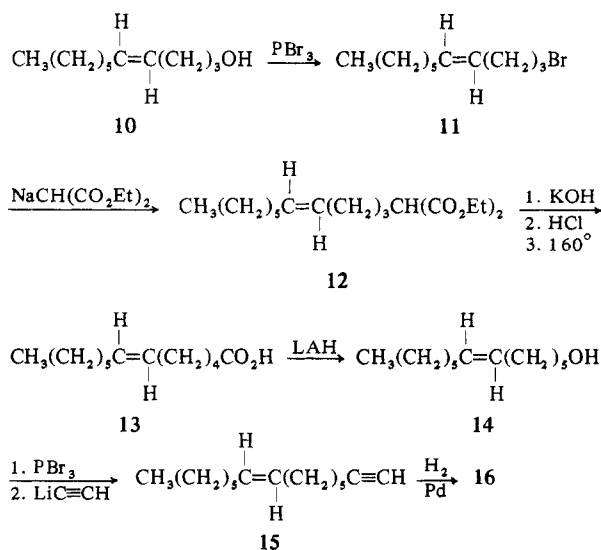


(3) to its tetrahydropyranyl ether (4), which was then treated with lithium acetylide-ethylenediamine complex in DMSO to form 2-(6-heptynyloxy)tetrahydropyran (5). Alkylation of 5 with 1-bromohexane and NaNH<sub>2</sub> in liquid NH<sub>3</sub> gave 2-(6-tridecyloxy)tetrahydropyran (6), which was hydrolyzed to 6-tridecyn-1-ol (7). Treatment of 7 with PBr<sub>3</sub> gave 1-bromo-6-tridecyne (8). The reaction, whether carried out in toluene or hexane with pyridine as a catalyst, gave only a moderate yield of 8 which seemed to be accompanied by some dibromo derivative and a dialkyl phosphite. The isolation of 8 was made very difficult by emulsion formation, a frequent problem when substances with 13–15 C atoms were being prepared.

Compd 8 was converted smoothly to 1,8-pentadecadiyne (9) by condensation with LiC≡CH. Complete reduction of 9 over PtO<sub>2</sub> gave pentadecane, as expected; controlled reduction with Lindlar catalyst (modified with quinoline) gave 2 which was identical in physical and chemical properties with the purified *Echinacea* diene.

(*E*)-1,8-Pentadecadiene (16) was prepared by the method shown in Scheme II. (*E*)-4-Undecen-1-ol (10)<sup>22</sup> was con-

Scheme II



verted to the corresponding bromide (11), which was condensed with diethyl sodiomalonate; hydrolysis and decarboxylation of the product (12) gave (*E*)-6-tridecenoic acid (13), which was reduced with LAH to (*E*)-6-tridecen-

Table I. Antitumor Activity of *Echinacea* and Related Olefins<sup>15,4</sup>

Material	Tumor system									
	FV <sup>b</sup>		LE <sup>c</sup>		PS <sup>c</sup>		SA <sup>b</sup>		WA <sup>b</sup>	
	Dose, mg/kg	% T/C	Dose, mg/kg	% T/C	Dose, mg/kg	% T/C	Dose, mg/kg	% T/C	Dose, mg/kg	% T/C
<i>E. pallida</i> (extract)			400	89	200	88			400	8-41
			200	105	50	94				
<i>E. angustifolia</i> (distd oil)			400	104	400	200			400	31
( <i>Z</i> )-C <sub>15</sub> H <sub>28</sub> (2)					100	127			400	14
( <i>E</i> )-C <sub>15</sub> H <sub>28</sub> (16)			400	104	150	122				
					75	90				
1-Tetradecene			350	92	265	100	500	82		
1-Pentadecene	400	115	400	86	50	100	500	84	400	17
1-Hexadecene	450	132	450	88	145	104	250	104		

<sup>a</sup>All tumors were in the mouse except WA, which was in the rat. <sup>b</sup>A material is considered active if it causes reduction of tumor weight (% T/C) to 42% or less. <sup>c</sup>A material is considered active if it causes increase of animal survival time to 25% or more (T/C = 125%).

1-ol (14). The bromide prepared from 14 was condensed with LiC≡CH to give (*E*)-8-pentadecen-1-yne (15), which was reduced catalytically to the desired 16. The product showed the expected ir spectrum, and glc retention times were identical with those from the trans portions of isomerized 2.

An attempt to reduce 9 with Na in liquid NH<sub>3</sub> gave only traces of 16, presumably because the acetylene formed an insoluble Na salt. The diyne 9 was, however, converted to 16 by replacing the terminal H with a trimethylsilyl group (*via* the Grignard derivative)<sup>23</sup> and reducing the internal triple bond with Li in EtNH<sub>2</sub><sup>24</sup> followed by removal of the Me<sub>3</sub>Si group with AgNO<sub>3</sub>-KCN.

The possibility of forming the trans diene 16 by altering the bond in 2 was also studied; some conversion was achieved, but the extent was small. Heating 2 with iodine or with HNO<sub>3</sub>-HNO<sub>2</sub> mixture<sup>25</sup> did not give detectable amounts of 16 (ir analysis). However, when the natural or synthetic cis diene was heated to a high temp in the presence of S or red P<sup>26</sup> or of Se,<sup>27</sup> as much as 11% was transformed into 16.

In an alternative synthesis of 16, the terminal CH<sub>2</sub> was introduced by using the Wittig olefin synthesis. Thus, 2-(7-tetradecynyloxy)tetrahydropyran (17) was cleaved by acid to 7-tetradecyn-1-ol (18), which was reduced to the trans olefinic alcohol (19). Heating the tosylate with DMSO gave (*E*)-7-tetradecenal (20), which could be converted to 16 by using a =CH<sub>2</sub>-yielding ylid.

**Biological Activity.** The results of biological tests with the natural products and with a number of synthetic olefins against several *in vivo* tumor systems<sup>18</sup> are shown in Table I. From the evaluation of the assay results in the P-388 lymphocytic leukemia (PS), L-1210 lymphoid leukemia (LE), Walker carcinosarcoma 256 (WA), Friend virus leukemia (solid) (FV), and Sarcoma-180 (SA) systems on a statistical basis in sequential testing, the distilled oil of roots of *E. angustifolia* was found to be active in PS and WA and inactive in LE. Compd 2 was likewise active in PS and WA; the trans isomer 16 showed borderline activity in PS and was inactive in LE. Of the olefins 1-tetradecene, 1-pentadecene, and 1-hexadecene, only 1-pentadecene (di-hydro-2) was active; it showed inhibition of the WA system.

The oncolytic activity of (*Z*)-1,8-pentadecadiene (2) is of a low order, but it is a promising lead in the possible synthesis of more active compounds, particularly since a diene olefin has not heretofore been reported to show antitumor activity.

## Experimental Section‡

**Isolation and Fractionation of *Echinacea* Oil.** The root oils of *E. angustifolia* and *E. pallida* were obtd by the pentane extn and MeCN partition method described previously.<sup>14</sup> The oil (50 g) from *E. angustifolia* was dild with hexane, washed with H<sub>2</sub>O to remove sticky, yellow semisolid, freed of solvent, and fractionally distd twice to give 22.2 g of colorless hydrocarbon fraction, bp 78-80° (0.3 mm), *n*<sub>D</sub><sup>25</sup> 1.4492; distn at 0.005 mm avoided some charring of the residue and gave the desired product at bp 56-60°. The hydrocarbon fraction from *E. pallida* oil distd at bp 80-82° (0.5 mm). Steam distn of the oils was extremely slow and impractical.

The distn residues were shown by column chromatog on Al<sub>2</sub>O<sub>3</sub> (Woelm neutral) to contain sterols, one of which had tlc properties similar to those of β-sitosterol and gave green colorations in the Liebermann-Burchard test.<sup>28</sup> These were not investigated further.

Chromatography of the hydrocarbon fraction (160 mg) on a column (2 × 24 cm) of silicic acid-25% AgNO<sub>3</sub> and elution with 5% Et<sub>2</sub>O in hexane gave 13 mg of a nearly odorless liquid identified by glc (DEGS column) as 1-pentadecene. Subsequent elution with 10% Et<sub>2</sub>O in hexane gave 118 mg of 2 as a colorless liquid: ir (film) 3070, 3005, 2930, 2862, 2820 (weak, probably overtone), 1645, 1460, 990, 910, 720 cm<sup>-1</sup>; nmr (CCl<sub>4</sub> with tetramethylsilane) ppm 0.9 (CH<sub>3</sub>), 1.2 (CH<sub>2</sub> chain), 1.97 and 2.06 (allylic CH<sub>2</sub>), 4.78-6.2 (overlapping multiplets; olefinic CH and CH<sub>2</sub>); mass spectrum showed the molecular ion at 208. *Anal.* (C<sub>15</sub>H<sub>28</sub>) C, H.

**Hydrogenation of 2.** Compd 2 (104 mg) in EtOH (7 ml) was shaken under H<sub>2</sub> at 29.5° in the presence of 5% Pd-CaCO<sub>3</sub> catalyst (50 mg). The uptake of H<sub>2</sub> was 24 ml (calcd for 2 double bonds, 22.4 ml). Evapn gave a colorless mobile oil, mp 8°, *n*<sub>D</sub><sup>25</sup> 1.4324, identical with authentic pentadecane, mp 8°, *n*<sub>D</sub><sup>25</sup> 1.4326, by glc (DEGS and SE 30) and ir.

**Oxidation and Ozonolysis of 2.** Oxidn of 2 with KMnO<sub>4</sub> and glc of the resulting liquid acid mixt on a column (0.32 × 305 cm) of silicized glass beads (80-120 mesh) supporting 0.4% isophthalic acid and 0.25% Carbowax 20M yielded equal amts of hexanoic and heptanoic acids with small amts of propionic and valeric acids. Soft waxy platelets, mp 47-49°, were identified as myristic acid by mixture mp and ir; further confirmation was obtd by glc (SE 30 on Chromosorb) of the Me esters (prepd with BF<sub>3</sub>-MeOH). Sublimation of the evapd filtrate sepd a small amt of adipic acid, mp 152°.

A soln of 208 mg of 2 in a mixt of 850 mg of 2,4-dinitrophenylhydrazine, 4.5 ml of H<sub>2</sub>SO<sub>4</sub>, 6 ml of H<sub>2</sub>O, and 25 ml of EtOH was ozonized for 50 min in a Bonner apparatus, and the resulting orange ppt of 2,4-dinitrophenylhydrazones was subjected to tlc on silica gel G. Development with C<sub>6</sub>H<sub>6</sub>-AcOEt (19:1) gave spots with R<sub>f</sub> 0.38-0.4 and 0.58-0.62 derived from HCHO and heptanal, respec-

‡Melting points were detd in a Mel-Temp apparatus, and are uncorrected. Boiling points are uncorrected. Ir spectra were detd with Perkin-Elmer Models 21, 137, and 521. Nmr spectra were recorded with a Varian T-60 spectrometer. Mass spectra were obtd with a Consolidated Electrodynamics Corp. Model 21-110B spectrometer. Where analyses are indicated only by symbols of the elements, analytical results obtd from those elements were within ±0.4% of the calcd values. Analyses were performed by Galbraith Laboratories, Knoxville, Tenn. The mention of a proprietary product in this paper does not constitute an endorsement of this product by the U. S. Department of Agriculture.

tively (comparison with authentic 2,4-dinitrophenylhydrazones).

**Hydrogenolysis of 2 Ozonides.** Injection at 295–300° of a soln of 2 ozonides in 2,2,4-trimethylpentane through a Pd catalytic reductor attachment onto a column of 5% squalane on Chromosorb P at 35° with H<sub>2</sub> as the carrier gas<sup>11</sup> gave pentane and hexane as the major products, as detd by glc.

**Stereochemistry of the Isolated Double Bond of 2.** Conversion of the C<sub>8</sub> double bond from cis to trans occurred when 2 was heated at 220° for several hr with S, red P, or gray Se. Although sepn of the mixed isomers was not possible by glc on Carbowax, the product showed a strong trans band at 965–966 cm<sup>-1</sup> on ir analysis.

**2-(5-Chloropentyloxy)tetrahydropyran (4).** A mixt of 74 g of 1-chloro-5-pentanol (3)<sup>8</sup> and 0.3 ml of concd HCl was treated with 50 g of freshly distd dihydropyran over 1.5 hr, stirred an addnl 3 hr at 20°, treated with 1 g of NaHCO<sub>3</sub>, and distd. After a small forerun, 102.9 g (82%) of 4 was obt'd as a colorless liquid, bp 63° (0.01 mm). *Anal.* (C<sub>10</sub>H<sub>19</sub>ClO<sub>2</sub>) C, H, Cl.

**2-(6-Heptynyloxy)tetrahydropyran (5).** A soln of 102.9 g (0.53 mole) of 4 in 150 ml of DMSO was slowly added, over 2 hr, to a stirred slurry of 54.6 g (0.6 mole) of lithium acetylide-ethylenediamine complex in 250 ml of dry DMSO cooled to 15°. The mixt was stirred an addnl 3 hr at 25° and dild with ice H<sub>2</sub>O, and the aqueous layer was extd with Et<sub>2</sub>O. The combined organic phase was washed with satd aqueous NaCl, dried, and distd to give 81.1 g (83%) of colorless 5, bp 72° (0.1 mm), *n*<sup>25</sup><sub>D</sub> 1.4584. *Anal.* (C<sub>15</sub>H<sub>24</sub>O<sub>2</sub>) C, H.

**2-(6-Tridecynyloxy)tetrahydropyran (6).** To a soln of 20 g of NaNH<sub>2</sub> in 1.5 l. of liquid NH<sub>3</sub> was added, dropwise with stirring, 79.5 g (0.405 mole) of 5. After the soln was stirred for an addnl 1.5 hr, 100 g (0.63 mole) of 1-bromohexane and 25 g (0.118 mole) of 1-iodohexane were slowly added, and the mixt was stirred another 5 hr. The NH<sub>3</sub> was allowed to evap, and 600 ml of ice H<sub>2</sub>O was added under N<sub>2</sub>; then the organic layer was extd with Et<sub>2</sub>O. The combined ext was washed with satd aqueous NaCl, dried, and freed of solvent. The crude 6 (70.6 g) was used without distn in the next step.

**6-Tridecyn-1-ol (7).** A stirred soln of 70.6 g of 6 in 600 ml of MeOH was treated dropwise with 160 ml of 12 *N* H<sub>2</sub>SO<sub>4</sub>, stirred an addnl 3 hr, and poured into 1200 ml of H<sub>2</sub>O. The mixt was extd with hexane, and the dried ext was distd. The product 7, bp 107–109° (0.1 mm), *n*<sup>25</sup><sub>D</sub> 1.4608, weighed 57.2 g (72% based on 5). *Anal.* (C<sub>13</sub>H<sub>24</sub>O) C, H.

**1-Bromo-6-tridecyn-1-ol (8).** A soln of 49 g (0.25 mole) of 7 in 100 ml of dry toluene was treated over 20 min at –8° with a soln of 30 g (0.11 mole) of PBr<sub>3</sub> in 15 ml of toluene; the mixt was stirred for 2 hr on the steam bath and poured into ice H<sub>2</sub>O. Extn with hexane in a liquid-liquid extractor followed by drying of the ext and distn gave 28.6 g (44%) of colorless 8, bp 102° (0.05 mm).

**1,8-Pentadecadiyne (9).** Treatment of 17.5 g of 8 with 8.2 g of lithium acetylide-ethylenediamine in DMSO, as described previously to furnish 5, gave the desired product (10.8 g; 77%) as a colorless liquid, bp 80° (0.05 mm), *n*<sup>25</sup><sub>D</sub> 1.4575. *Anal.* (C<sub>15</sub>H<sub>24</sub>) C, H.

**(Z)-1,8-Pentadecadiene (2).** Compd 9 (10 g) in abs EtOH was hydrogenated at 26° by using 2.3 g of Lindlar catalyst to which 0.5 g of quinoline had been added. Total absorption of H<sub>2</sub> was 2275 ml, the theor vol being about 2190 ml. The catalyst was filtered off, the EtOH was evapd, and the product was dissolved in hexane and washed free of quinoline with dil acid and H<sub>2</sub>O. Distn of the dried soln yielded 8.43 g (84%) of 2, bp 78.5° (0.05 mm), *n*<sup>25</sup><sub>D</sub> 1.4480. *Anal.* (C<sub>15</sub>H<sub>28</sub>) C, H.

Synthetic 2 showed ir and nmr spectra identical with those obt'd with natural 2. Heating 350 mg of synthetic 2 with 550 mg of 2,4-dinitrobenzenesulfonyl chloride in 11 ml of AcOH at 100° for 1 hr and pouring the cooled mixt on ice gave 784 mg of yellow oil, from which 180 mg of *adduct* was obt'd by chromatography on a column of Merck silicic acid [elution with hexane followed by elution with C<sub>6</sub>H<sub>6</sub>-hexane (2:3)]. The *adduct* was identical (ir) with that prep'd from natural 2. *Anal.* (C<sub>21</sub>H<sub>31</sub>ClN<sub>2</sub>O<sub>4</sub>S) Cl.

**(E)-1-Bromo-4-undecene (11).** A soln of 48 g of (*E*)-4-undecen-1-ol<sup>12</sup> (10) in toluene was treated with 28 g of PBr<sub>3</sub> under the conditions described for prep'd 8. The toluene soln was washed, dried, and distd to yield 37.9 g (58%) of 11, bp 125–133° (14 mm), 57–60° (0.05 mm), *n*<sup>25</sup><sub>D</sub> 1.4627.

**(E)-6-Tridecenoic Acid (13).** Compd 11 (34.7 g) was condensed with 24 g of diethyl malonate and 3.6 g of Na in 80 ml of BuOH by refluxing at 90° for 1 hr. The ppt of NaBr was filtered off, and the filtrate was dild with H<sub>2</sub>O and freed of BuOH by distn. The residual

diester 12 was saponified by boiling for 4 hr with a soln of 21 g of KOH in 25 ml of H<sub>2</sub>O, and the mixt was acidified with concd HCl and extd with several portions of C<sub>6</sub>H<sub>6</sub> and hexane. Evapn of the solvent gave crude (*E*)-4-undecenylmalonic acid, mp 72°, which was decarboxylated by heating at 160° until CO<sub>2</sub> evoln ceased. Distn gave 20.0 g (63%) of colorless 13, bp 138–142° (0.25 mm). *Anal.* (C<sub>13</sub>H<sub>24</sub>O<sub>2</sub>) C, H; neut equiv 218.7, calcd 212.3.

**(E)-6-Tridecen-1-ol (14).** Reduction of 13 (19.5 g) in the usual way with LAH (4.75 g) gave a 66% yield of 14, bp 117–118° (0.4 mm), *n*<sup>25</sup><sub>D</sub> 1.4532. *Anal.* (C<sub>13</sub>H<sub>26</sub>O) C, H.

**(E)-8-Pentadecen-1-yne (15).** Method A. Treatment of 14 (12.4 g) with PBr<sub>3</sub> (6.6 g) in the manner described previously gave a 63% yield of colorless (*E*)-1-bromo-6-tridecene, bp 95–96° (0.01 mm), *n*<sup>25</sup><sub>D</sub> 1.4678.

Treatment of 10.1 g of the bromide with 4 g of lithium acetyl-ideethylenediamine in DMSO, as described previously to obtain 5, gave 5.6 g (70%) of 15, bp 142–143° (15 mm), ir (film) 3300 (C≡CH), 2128 (C≡C), and 970 cm<sup>-1</sup> (*trans*-CH=CH). *Anal.* (C<sub>15</sub>H<sub>26</sub>) C, H.

Method B. Commercial MeMgCl (8 ml of 3.1 *N* in THF) was added during 30 min at room temp to 4.1 g of comp'd 9 in 25 ml of THF followed, after 10 min, by the slow addn of a soln of 2.75 g of chlorotrimethylsilane in 10 ml of THF. After addition was complete, the mixt was stirred for 15 min, poured into 100 ml of satd NH<sub>4</sub>Cl, and extd with hexane. Distn of the dried ext gave 0.6 g of unchanged 9 and 2.63 g (48%) of trimethylsilyl-1,8-pentadecadiyne, bp 118–119° (0.1 mm), *n*<sup>25</sup><sub>D</sub> 1.4608. *Anal.* (C<sub>18</sub>H<sub>32</sub>Si) C, H, Si.

A soln of 0.24 g of Li in 35 ml of Et<sub>2</sub>NH was treated slowly at –78° with 3.7 g of the trimethylsilyl derivative, stirred for 2 hr, allowed to stand overnight to evap the amine, decomp'd with H<sub>2</sub>O, and extd with hexane. The ext was freed of solvent, taken up in EtOH, and treated with a soln of 1.35 g of AgNO<sub>3</sub> in 75% EtOH. The creamy acetylide ppt was washed, resuspended in aqueous EtOH, and treated with 2.2 g of NaCN in 4 ml of H<sub>2</sub>O. The resulting oily layer was chromatog'd on a silicic acid column; comp'd 15 eluted preferentially with hexane.

**(E)-1,8-Pentadecadiene (16).** A soln of 5.5 g of 15 in 30 ml of hexane contg 0.5 ml of EtOH absorbed 660 ml of H<sub>2</sub> in 25 min when a catalyst was used composed of 0.15 g of 5% Pd–CaCO<sub>3</sub> and 0.05 g of 5% Pd–BaSO<sub>4</sub>. Filtration of the catalyst, evapn of the solvent, and distn of the resulting oil gave 4 g of 16, bp 138–140° (16 mm). The product cont'd a little pentadecane, which was removed with hexane from a column of silicic acid contg 25% AgNO<sub>3</sub>; pure 16, *n*<sup>25</sup><sub>D</sub> 1.4458, was eluted with 3% Et<sub>2</sub>O in hexane; ir (film) 3077, 2915, 2857, 1824, 1650, 1465, 1445, 1380, 1304, 1260, 994, 967, 909, 725 cm<sup>-1</sup>. *Anal.* (C<sub>15</sub>H<sub>28</sub>) C, H.

**2-(7-Tetradecynyloxy)tetrahydropyran (17).** 2-(7-Octynyloxy)-tetrahydropyran<sup>29</sup> (9 g, 0.043 mole) was added slowly, under N<sub>2</sub>, to a suspension of LiNH<sub>2</sub> (1.25 g) in dry freshly distd (over Na) dioxane (55 ml), and the mixt was refluxed for 3 hr, cooled, and treated dropwise with 1-bromohexane (8.1 g, 0.05 mole). Refluxing was maintained for 17 hr, and the mixt was then cooled, decomp'd with H<sub>2</sub>O, and extd with Et<sub>2</sub>O. The dried ext was freed of solvent and distd twice to give 4.59 g (60%) of product, bp 140° (0.1 mm). *Anal.* (C<sub>15</sub>H<sub>34</sub>O<sub>2</sub>) C, H.

**7-Tetradecyn-1-ol (18).** Acid hydrolysis of 17 by the procedure used to obtain 7 gave 18, bp 104–105° (0.01 mm), *n*<sup>25</sup><sub>D</sub> 1.4627, in 81% yield.

**(E)-7-Tetradecen-1-ol (19).** Reduction of 18 with H<sub>2</sub> in the presence of Pd–CaCO<sub>3</sub> catalyst gave 19, bp 100–105° (0.01 mm), in 67% yield. This was used without analysis for the next step.

**(E)-7-Tetradecenal (20).** A mixt of 1.5 g of 19, 1.6 g of TsCl, and 8 ml of pyridine was stirred for 3 hr at 20°, decomp'd with ice and concd HCl, and extd with Et<sub>2</sub>O. The crude product (1.8 g, 58%), which became cryst at –15°, was added to a stirred mixt of 30 ml of DMSO and 3.7 g of NaHCO<sub>3</sub> at 150°, cooled after 3 min, dild with ice H<sub>2</sub>O, and extd with hexane. Distn of the resulting oil gave 20 (0.4 g), bp 88–93° (0.05 mm).

**Wittig Condensation of Comp'd 20.** An Et<sub>2</sub>O soln of 0.3 g of 20 was added with stirring to an Et<sub>2</sub>O soln contg 2.4 ml of 1.6 *M* BuLi in hexane and 0.8 g of the ylid from methyltriphenylphosphonium iodide. Stirring was continued for 30 min, the ppt was filtered off, and the filtrate was evap'd to give 0.19 g of crude 16. Chromatog-raphy on a column of silicic acid contg 25% AgNO<sub>3</sub>, by successive elution with hexane and with 4% and 8% Et<sub>2</sub>O–hexane, gave pure 16 in the last eluate.

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## Synthesis of the Luteinizing-Releasing Hormone of the Hypothalamus and the 8-Lysine Analog†

Jaw-Kang Chang, Hans Sievertsson, Bruce L. Currie, Conny Bogentoft, Karl Folkers,\*

*Institute for Biomedical Research, The University of Texas at Austin, Austin, Texas 78712*

and Cyril Y. Bowers

*Tulane University, School of Medicine, New Orleans, Louisiana 70112. Received November 21, 1971*

An advantageous synthesis of the hypothalamic-releasing hormone (LRH), which is pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub>, has been achieved by "classical reactions." In principle, the 1-6 sequence, pGlu-His-Trp-Ser-Tyr-Gly, and the 7-10 sequence, Leu-Arg-Pro-Gly, are separately synthesized and then coupled to give the decapeptide. There were appropriate protective groups; good yields, helpful stepwise purifications, and new improvements are evident. Since the Arg<sup>8</sup> moiety of the decapeptide is presumably important to hormonal activity and potency, this new synthesis provides the flexibility needed to replace Arg<sup>8</sup> with new moieties in the relatively small 7-10 sequence. For example, this synthesis was modified to obtain the 8-lysine-luteinizing-releasing hormone. Lys<sup>8</sup>-LRH (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Lys-Pro-Gly-NH<sub>2</sub>) was found to release significant levels of the luteinizing hormone in the rat assay, but at nanogram dose levels which were not much higher than that required for LRH. Thus, the relative importance of a guanidino group over an amino group in the 8-moiety for potent activity is evident.

Sievertsson, *et al.*,<sup>1</sup> and Folkers‡<sup>2</sup> described our first 2 syntheses of the luteinizing- (hormone) releasing hormone of the hypothalamus which is the decapeptide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub>. In the first synthesis, the tripeptide, pGlu-His-Trp, was obtained by "classical reactions" and then coupled with the protected heptapeptide corresponding to Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub> which had been separately synthesized by the Merrifield solid-phase technique. The second synthesis was entirely by solid-phase coupling. The decapeptides from

both syntheses were identical according to the results of extensive countercurrent distribution, hormonal assays, and other comparative data. This decapeptide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub>, is the hypothalamic-releasing hormone (LRH) which regulates the luteinizing hormone of the anterior pituitary gland.

The background for these 2 syntheses of LRH was described so recently<sup>1</sup> that it is not repeated herein, but citations to synthesis of the first LRH-active peptide, pGlu-Tyr-Arg-Trp-NH<sub>2</sub>, by Chang, *et al.*,<sup>3</sup> and a synthesis of LRH by a solid-phase procedure described by Monahan, *et al.*,<sup>4</sup> are particularly relevant.

We described our third synthesis of the luteinizing-re-

†Hypothalamic Hormones. 36.

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