

## A Practical Synthesis of the Anti-tumour Agent 2-Methyl-4-deoxyisopropodophyllotoxin and Related Podophyllin Analogues

Andrew R. Beard, Sarah J. Hazell, John Mann\* and Christopher Palmer  
Chemistry Department, Reading University, Whiteknights, Reading RG6 2AD, UK

We describe an efficient, six-stage synthesis of 2-methyl-4-deoxyisopropodophyllotoxin, which proceeds *via* a Diels–Alder cycloaddition between 2-methylmaleic anhydride and the orthoquinodimethane generated by treatment of 5-[1-acetoxy-1-(3',4',5'-trimethoxyphenyl)methyl]-6-trimethylsilylmethyl-1,3-benzodioxole with silica gel at 60 °C. The podophyllin was converted into a number of 4'-ester derivatives. This procedure was also used to prepare a diazapodophyllin *via* the use of 4-phenyl-1,2,4-triazoline-3,5-dione as dienophile.

Extracts of the plants *Podophyllum peltatum* and *P. emodii* have long been valued for the treatment of warts and other benign tumours, and their major constituents, the aryltetralin lignans podophyllotoxin **1** and beta-peltatin **2**, have proven activity as inhibitors of cell division.<sup>1</sup> Two semi-synthetic derivatives of **1**, etoposide **3** and teniposide **4**, are now widely and successfully used for the treatment of, *inter alia*, small cell lung cancer, testicular teratoma and refractory childhood leukaemia.<sup>2</sup>

In 1985 we described a route to 2-substituted-4-deoxy-analogues of podophyllotoxin **5** which proceeded *via* cycloaddition reactions between the orthoquinodimethane **6** and dienophiles **7** (Scheme 1).<sup>3</sup> We went on to show that several of these analogues, most notably **5a**, exhibited marked *in vivo* anti-tumour activity<sup>4</sup> despite possessing a *cis*-lactone ring rather than the *trans*-ring of podophyllotoxin. Computer modelling of the various X-ray structures for analogues **5** demonstrated that they all possessed very similar three-dimensional structures to podophyllotoxin. In particular, the pendant aryl rings were perpendicular to the rather flattened ABCD ring systems, a structural feature that appears to be important for anti-mitotic activity.<sup>5</sup>

The route shown in Scheme 1 could not, however, be used for the large-scale synthesis of podophyllins. In order to overcome this problem, we devised a milder and more practical route to aryltetralin lignans of this type, and the overall route is shown in Scheme 2. During the course of this work, Takano and co-workers reported a conceptually similar route (shown in Scheme 3),<sup>6</sup> but a full account of this work has not yet appeared, so the practical utility of the methodology cannot be assessed. In addition, our method employs silica as the 'trigger' for the cycloaddition, whereas the Japanese workers used the dienophile maleic anhydride as 'trigger'.

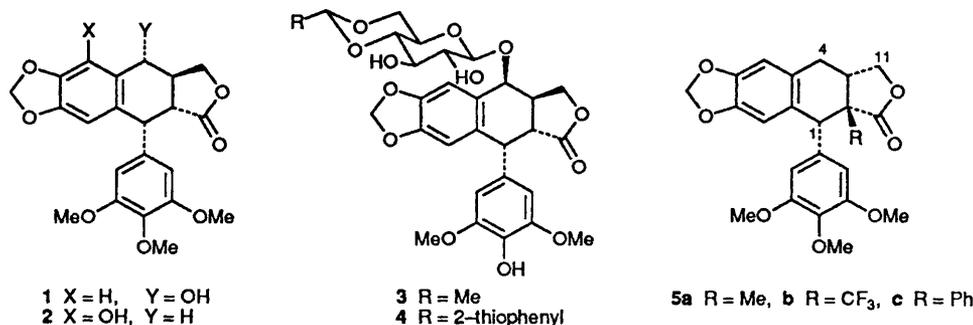
The requisite orthoquinodimethane precursor **12** was prepared in four steps from piperonyl bromide **8**. Reaction of this with magnesium turnings (previously activated by vigorous overnight stirring under nitrogen)<sup>7</sup> in the presence of trimethylsilyl chloride, provided the silane **9**, which was

immediately brominated to yield the bromo silane **10** (27% overall yield for the two steps on the 0.4 molar scale). Metal-halogen exchange was effected with butyllithium and the resultant aryllithium was treated with 3,4,5-trimethoxybenzaldehyde to yield the alcohol **11** (50–70% on the 0.1 molar scale). This was then acetylated to provide the desired orthoquinodimethane precursor **12**.

We envisaged that this would react with fluoride ion to produce the orthoquinodimethane **6**, but attempts to achieve this transformation with tetrabutylammonium fluoride and caesium fluoride in the presence of the dienophile 2-methylmaleic anhydride (citraconic anhydride) produced intractable mixtures of products. However, during attempted purification of the acetate **12** on silica gel, we noticed a blue coloration on the column, and subsequently were able to isolate an inseparable mixture of the two compounds **13** and **14** in the eluent. These are products of intramolecular cycloaddition of the required orthoquinodimethane **6**. Presumably the silica acts as both Lewis acid and source of nucleophiles. This serendipitous observation coupled with some process development led to the successful methodology shown in Scheme 2. The reaction with citraconic anhydride has been carried out routinely and reproducibly on the 30 mmol scale using this methodology to yield the cycloadduct **15** in yields of 70–75% after chromatography. Much smaller amounts (never greater than 10%) of the alternative regioisomer **16** were also isolated.

There has been considerable interest recently in the preparation of aza- and diaza-podophyllins, *e.g.* **17**<sup>8</sup> and **18**,<sup>9</sup> and it was thus of interest to attempt cycloadditions with diaza-dienophiles. Under our standard reaction conditions we could not obtain any cycloadducts with diethyl azodicarboxylate, but use of 4-phenyl-1,2,4-triazoline-3,5-dione **19** provided the cycloadduct **20** in 28% isolated yield.

Finally, in order to extend our knowledge of the structure-biological activity profiles of these podophyllin analogues, we converted the cycloadduct **15** into lactone **5a** using K-selectride in THF followed by treatment with acid.<sup>4</sup> This lactone could be





Reactions requiring anhydrous conditions were carried out under a static nitrogen atmosphere using oven-dried glassware. THF (tetrahydrofuran) was dried over molecular sieves and distilled from sodium–benzophenone prior to use. All other reagents and solvents were used as supplied without further purification. Flash chromatography was performed using Sorbosil C60 silica gel. Ether refers to diethyl ether and light petroleum refers to the fraction with b.p. 40–60 °C unless otherwise stated.

**Piperonyl Bromide 8.**—Piperonyl alcohol (101.44 g, 0.067 mol) was added portionwise to HBr (49% aq., 100 cm<sup>3</sup>), and the resultant suspension was allowed to stand at room temp. for 2 h. Dichloromethane (1 dm<sup>3</sup>) was then added, and the organic layer separated. The aqueous layer was extracted with dichloromethane (3 × 800 cm<sup>3</sup>), and the combined organic extract was washed with water (2 × 500 cm<sup>3</sup>) and saturated aqueous sodium hydrogen carbonate (3 × 500 cm<sup>3</sup>), dried and concentrated to yield the crude bromide (123.63 g, ca. 85%), which was used without further purification. An analytical sample was prepared by recrystallisation from light petroleum, m.p. 46–48 °C (lit.,<sup>3</sup> m.p. 48 °C);  $\delta$ (CDCl<sub>3</sub>; 60 MHz) 4.43 (s, 2 H, CH<sub>2</sub>Br), 5.9 (s, 2 H, OCH<sub>2</sub>O) and 6.8 (m, 3 H, ArH).

**5-Bromo-6-trimethylsilylmethyl-1,3-benzodioxole 10.**—Magnesium turnings (9.04 g, 0.38 mol) were activated by stirring overnight under a stream of nitrogen. Freshly distilled THF (75 cm<sup>3</sup>) and freshly distilled trimethylsilyl chloride (48 cm<sup>3</sup>, 0.038 mol) were added, followed by the dropwise addition of a solution of piperonyl bromide (73.62 g, 0.38 mol) in THF (175 cm<sup>3</sup>) over a period of 1 h at 0 °C. After the addition was complete, the reaction mixture was poured into an ice–water mixture (1 dm<sup>3</sup>), and the products were extracted with light petroleum (2 × 500 cm<sup>3</sup>). The combined organic extract was dried and concentrated to yield the crude silane **9** (44.93 g). This was dissolved in dichloromethane (200 cm<sup>3</sup>), and to this was added aqueous sodium carbonate (10%, 200 cm<sup>3</sup>), followed by bromine (14 cm<sup>3</sup>, 0.27 mol) in dichloromethane (50 cm<sup>3</sup>), and the mixture held at 0 °C for 1 h. The organic layer was separated off, and the aqueous layer extracted with dichloromethane (2 × 200 cm<sup>3</sup>). The combined organic extract was dried, concentrated, and the products separated by flash chromatography, eluting with light petroleum–ether (9:1) to yield the bromo silane **10** (25.87 g, 27% overall for two steps). *R*<sub>f</sub> 0.75 (light petroleum–ether, 9:1);  $\delta$ (CDCl<sub>3</sub>; 60 MHz) 0.0 (s, 9 H, SiMe<sub>3</sub>), 2.15 (s, 2 H, CH<sub>2</sub>TMS), 5.82 (s, 2 H, OCH<sub>2</sub>O), 6.5 (s, 1 H, 7-H) and 6.85 (s, 1 H, 4-H); *m/z* 288.

**5-[1-Acetoxy-1-(3',4',5'-trimethoxyphenyl)methyl]-6-trimethylsilylmethyl-1,3-benzodioxole 12.**—Butyllithium (40 cm<sup>3</sup> of a 2.5 mol dm<sup>-3</sup> solution in hexane; 0.1 mol) was added dropwise to a stirred solution of the bromo silane **10** (25.39 g, 0.092 mol) in dry THF (400 cm<sup>3</sup>) over a 40 min period with stirring at –78 °C. The resultant solution was stirred at the same temperature for 2.5 h prior to the dropwise addition of 3,4,5-trimethoxybenzaldehyde (25.05 g, 0.13 mol) in dry THF (100 cm<sup>3</sup>). The mixture was then warmed to room temp. and stirred overnight. After addition of water (100 cm<sup>3</sup>), the THF was removed under reduced pressure, and the residue was diluted with water (200 cm<sup>3</sup>), and the products extracted into dichloromethane (3 × 250 cm<sup>3</sup>). The combined organic extract was washed with brine (200 cm<sup>3</sup>), dried and concentrated to yield the crude alcohol **11** (ca. 18 g, around 50%). The yield was increased to 70% when the reaction was carried out on an 18 g scale. The alcohol (15.11 g, 37 mmol) was dissolved in pyridine (50 cm<sup>3</sup>) and acetylated with acetic anhydride (3.62 cm<sup>3</sup>, 44.5 mol) at 0 °C. The reaction was complete after 24 h at room temp., and the mixture was partitioned between water (150 cm<sup>3</sup>)

and light petroleum (150 cm<sup>3</sup>). The organic layer was separated and the aqueous layer was extracted with light petroleum (2 × 150 cm<sup>3</sup>). The combined organic extract was washed with 2 mol dm<sup>-3</sup> HCl (2 × 150 cm<sup>3</sup>), brine (150 cm<sup>3</sup>), dried and concentrated to yield the acetate **12**. Recrystallisation from light petroleum provided pure product (9.85 g, 59%). *R*<sub>f</sub> 0.4 (light petroleum–ether 2:1); m.p. 99–101 °C;  $\nu_{\max}$ /cm<sup>-1</sup> 2955, 2865, 2839, 1728, 1598, 1502, 1482, 1369, 1235, 1019, 869 and 692;  $\delta$ (CDCl<sub>3</sub>; 220 MHz) 0.0 (s, 9 H, Me<sub>3</sub>Si), 2.05 (s, 2 H, CH<sub>2</sub>TMS), 2.15 (s, 3 H, OAc), 3.8 (s, 6 H, 3'-OMe and 5'-OMe), 3.82 (s, 3 H, 4'-OMe), 5.95 (s, 2 H, OCH<sub>2</sub>O), 6.5 (s, 1 H, CHO), 6.52 (s, 2 H, 2'-H and 6'-H), 6.85 (s, 1 H, 7-H) and 6.9 (s, 1 H, 4-H) (Found: C, 61.85; H, 6.75. C<sub>23</sub>H<sub>30</sub>O<sub>7</sub>Si requires C, 61.89; H, 7.01%).

**3a,4-trans-3a,9a-cis-3a-Methyl-6,7-methylenedioxy-4-(3',4',5'-trimethoxyphenyl)-3a,4,9a-tetrahydronaphtho[2,3-c]furan-1,3-dione 15.**—A mixture of the acetate **12** (12.58 g, 28.2 mmol), citraconic anhydride (6.34 g, 56.57 mmol) and flash silica (10 g) was stirred in CCl<sub>4</sub> (200 cm<sup>3</sup>) at 50 °C for 10 d. The resultant coloured solution was filtered through Celite, then partitioned between water (250 cm<sup>3</sup>) and dichloromethane (250 cm<sup>3</sup>). The organic layer was separated, and the aqueous layer extracted with dichloromethane (2 × 200 cm<sup>3</sup>). The combined organic extract was washed with brine (200 cm<sup>3</sup>), dried, and concentrated. The residue was purified by chromatography using ether–light petroleum (2:1) as eluent to yield the desired cycloadduct **15** (9.17 g, 76%); *R*<sub>f</sub> 0.3 (light petroleum–ether, 2:1); m.p. 153 °C (lit.,<sup>3</sup> m.p. 153 °C);  $\nu_{\max}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 2900, 1850, 1785, 1590, 1485, 1460, 1330, 1230, 1125, 1040, 1005, 960 and 865;  $\delta$ (CDCl<sub>3</sub>; 400 MHz) 1.42 (s, 3 H, Me), 3.10 (dd, 1 H, *J* 15.9 and 9.8, 9<sub>alpha</sub>-H), 3.18 (m, 1 H, 9a-H), 3.40 (dd, 1 H, *J* 15.9 and 3.2, 9<sub>beta</sub>-H), 3.75 (s, 6 H, 3'-OMe and 5'-OMe), 3.80 (s, 3 H, 4'-OMe), 3.90 (s, 1 H, 4-H), 5.90 (s, 2 H, OCH<sub>2</sub>O), 6.32 (s, 2 H, 2'-H and 6'-H), 6.58 (s, 1 H, 5-H) and 6.75 (s, 1 H, 8-H) (Found: C, 64.95; H, 5.25. C<sub>23</sub>H<sub>22</sub>O<sub>8</sub> requires C, 64.77; H, 5.20%).

**2-Methyl-4-deoxyisodeoxypicropodophyllotoxin 5a.**—K-Selec-tride (22.52 cm<sup>3</sup> of a 1 mol dm<sup>-3</sup> solution in THF, 22.5 mmol) was added dropwise to a stirred solution of the cycloadduct **15** (4.80 g, 11.3 mmol) in dry THF (80 cm<sup>3</sup>) at –78 °C, and the resultant solution was stirred at –78 °C for 4.5 h. The reaction was quenched by the addition of water (12 cm<sup>3</sup>), and after warming to room temp., 2 mol dm<sup>-3</sup> NaOH (56 cm<sup>3</sup>) was added followed by CAREFUL addition of hydrogen peroxide (34 cm<sup>3</sup>, 30% solution) and the mixture was then stirred at room temp. overnight. 6 Mol dm<sup>-3</sup> HCl (34 cm<sup>3</sup>) was then added, and the solution was stirred for 15 min at room temp., and the products extracted into dichloromethane. This extract was dried, concentrated, and the residue taken up in THF (50 cm<sup>3</sup>), prior to the addition of DCC (2.95 g). The solution was then heated under reflux for 3 h, prior to chromatography (ether–light petroleum, 2:1 as eluent) which provided the desired lactone **5a** (3.72 g, 80%); *R*<sub>f</sub> 0.25 (ether–light petroleum, 2:1); m.p. 109–110 °C (lit.,<sup>4</sup> m.p. 108 °C);  $\nu_{\max}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 2900, 1765, 1590, 1505, 1485, 1330, 1230, 1125 and 1040;  $\delta$ (CDCl<sub>3</sub>; 400 MHz) 1.3 (s, 3 H, Me), 2.75 (m, 2 H, 3-H and 4<sub>alpha</sub>-H), 3.0 (m, 1 H, 4<sub>beta</sub>-H), 3.35 (dd, 1 H, *J* 10, 11<sub>beta</sub>-H), 3.71 (s, 6 H, 3'-OMe and 5'-OMe), 3.77 (s, 3 H, 4'-OMe), 3.93 (s, 1 H, 1-H), 4.25 (m, 1 H, *J* 10, 11<sub>alpha</sub>-H), 5.92 (s, 2 H, OCH<sub>2</sub>O), 6.41 (s, 2 H, 2'-H and 6'-H), 6.59 (s, 1 H, 8-H) and 6.71 (s, 1 H, 5-H) (Found: C, 66.97; H, 5.82. C<sub>23</sub>H<sub>24</sub>O<sub>7</sub> requires C, 66.97; H, 5.87%).

**2-Methyl-4-deoxy-4'-demethylisopicropodophyllotoxin 21.**—HBr was passed through a solution of the lactone **5a** (5.52 g, 13.4 mmol) in 1,2-dichloroethane–ether (10:1, 110 cm<sup>3</sup>) at 0 °C. The resultant solution was allowed to stand at 4 °C for

3 d, prior to concentration and chromatography (ether–light petroleum, 3:1 as eluent) to yield the desired phenol **21** (2.84 g, 53%);  $R_f$  0.25 (ether–light petroleum, 2:1); m.p. 170–174 °C;  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3536, 2965, 1759, 1616, 1516, 1481, 1424, 1325, 1218, 1116, 1021, 938, 861, 771 and 638;  $\delta(\text{CDCl}_3)$ ; 400 MHz) 1.32 (s, 3 H, Me), 2.72 (m, 2 H, 3-H and 4<sub>alpha</sub>-H), 3.03 (m, 1 H,  $J$  8.6, 4<sub>beta</sub>-H), 3.28 (dd,  $J$  8.8, 11<sub>beta</sub>-H), 3.79 (s, 6 H, 3'-OMe and 5'-OMe), 3.98 (s, 1 H, 1-H), 4.25 (dd, 1 H,  $J$  8.6, 11<sub>alpha</sub>-H), 5.42 (br s, 1 H, OH), 5.76 (s, 2 H, OCH<sub>2</sub>O), 6.42 (s, 2 H, 2'-H and 6'-H), 6.62 (s, 1 H, 8-H) and 6.72 (s, 1 H, 5-H) (Found: C, 65.5; H, 5.6. C<sub>22</sub>H<sub>22</sub>O<sub>7</sub> requires C, 65.58; H, 5.62%).

7,8-Methylenedioxy-2-phenyl-5-(3',4',5'-trimethoxyphenyl)-5,10-dihydro[1,2,4]triazolo[1,2-b]phthalazine-1,3(2H)-dione **20**.—4-Phenyl-1,2,4-triazoline-3,5-dione **19** (0.39 g, 2.2 mmol) was added to a stirred solution of the acetate **12** (0.5 g, 1.1 mmol) in carbon tetrachloride (10 cm<sup>3</sup>) containing suspended silica. The resultant suspension was heated at 50 °C for 4 d. The reaction mixture was partitioned between water (50 cm<sup>3</sup>), and dichloromethane (3 × 50 cm<sup>3</sup>), and the combined organic extract was washed once with water (50 cm<sup>3</sup>), dried and concentrated to yield an orange oil. This was purified by flash chromatography eluting with ether–light petroleum (2:1) to yield the cycloadduct **20** as a pale yellow foam (0.15 g, 28%);  $\nu_{\max}(\text{CDCl}_3)/\text{cm}^{-1}$  2983, 1773, 1712, 1594, 1503, 1457, 1422, 1265, 1163, 1042, 936 and 851;  $\delta(\text{CDCl}_3)$ ; 220 MHz) 1.95 (d, 1 H,  $J$  18, 10-H), 2.3 (d, 1 H,  $J$  18, 10-H), 3.75 (s, 6 H, 3'- and 5'-OMe), 3.85 (s, 3 H, 4'-OMe), 5.9 (d, 2 H,  $J$  2, OCH<sub>2</sub>O), 6.4 (s, 2 H, 2'- and 6'-H), 6.49 (s, 1 H, 5-H), 6.5 (s, 1 H, 6-H), 6.65 (s, 1 H, 9-H) and 7.4–7.5 (m, 5 H, Ph) (Found: M<sup>+</sup>, 489.1536. C<sub>26</sub>H<sub>23</sub>N<sub>3</sub>O<sub>7</sub> requires M, 489.1535).

#### Acknowledgements

We thank Food, Drugs, and Cosmetic Colours Ltd., for Ph.D.

studentships held by A. R. B. and S. J. H., and the Cancer Research Campaign for a Postdoctoral Fellowship held by C. P.

#### References

- J. D. Loike, *Trends Pharm. Sci.*, 1984, 30; I. Jardine in *Anticancer Agents based on Natural Product Models*, eds. J. M. Cassidy and J. D. Douros, Academic Press, New York, 1980, ch. 9.
- B. F. Issel, A. R. Rudolph, A. C. Louie and T. W. Doyle in *Etoposide (VP-16): Current Status and New Developments*, eds. B. F. Issel, F. M. Muggia and S. K. Carter, Academic Press, New York, 1984, chs. 1 and 2.
- J. Mann, S. E. Piper and L. K. P. Leung, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2081.
- A. R. Beard, M. G. B. Drew, J. Mann and L. T. F. Wong, *Tetrahedron*, 1987, 43, 4207.
- A. R. Beard, M. G. B. Drew, P. Hilgard, B. D. Hudson, J. Mann, S. Neidle and L. T. F. Wong, *Anti-Cancer Drug Design*, 1987, 2, 247.
- S. Takano, S. Otaki and K. Ogasawara, *J. Chem. Soc., Chem. Commun.*, 1985, 485.
- K. V. Baker, J. M. Brown, N. Hughes, A. J. Skarnulis and A. Sexton, *J. Org. Chem.*, 1991, 56, 698.
- H. L. Pearce, N. J. Bach and T. L. Cramer, *Tetrahedron Lett.*, 1989, 907; J.-P. Bosmans, J. Van der Eycken, M. Vandewalle, A. Hulkenberg, R. Van Hes and W. Veerman, *Tetrahedron Lett.*, 1989, 3877.
- J. Van der Eycken, J.-P. Bosmans, D. Van Haver, M. Vandewalle, A. Hulkenberg, W. Veerman and R. Nieuwenhuizen, *Tetrahedron Lett.*, 1989, 3873.
- M. Kuhn, C. Keller-Juslen and A. von Wartburg, *Helv. Chim. Acta*, 1969, 52, 944.
- R. S. Ward, *Synthesis*, 1992, 719.

Paper 3/00189J

Received 12th January 1993

Accepted 23rd February 1993