Chemistry of Natural Compounds, Vol. 46, No. 6, 2011

TROFIMOV SYNTHESIS OF BETULIN DERIVATIVES WITH 2,3-ANNELATED PYRROLE

A. V. Orlov,[1] G. R. Khazipova,[2] N. G. Komissarova,[2*] O. V. Shitikova,[2] L. V. Spirikhin,[2] and M. S. Yunusov[2]

UDC 547.91+547.759+547.314.2+547.288.4

*Lupane-type triterpenoid derivatives containing a 2,3-annelated 1*H*-pyrrole or an* N*-vinylpyrrole moiety were synthesized by reaction of 28-*O*Tr-lup-20(29)-en-3-one oxime and acetylene in KOH/DMSO superbase medium at atmospheric pressure.*

Keywords: betulin, acetylene, Trofimov reaction, pyrrole, *N*-vinylpyrrole.

Annelation of *N*-containing heterocycles with the triterpene skeleton is a promising and developing method for modifying pentacyclic triterpenoids of the lupane and oleanane types, natural compounds with broad spectra of biological activity [1].

Annelated structures are usually synthesized using classical methods. Pyrazine and quinoxaline derivatives of lupane- and oleanane-type triterpenoids were prepared at various times by condensation of triterpene α-diketones, α-*gem*-dibromo- or α-bromoketones with ethylene- or *o*-phenylenediamines [2–5]. Pyrazolo-triterpenoid structures were synthesized by condensation of β-diketones with *R*-hydrazines (*R* = H, Me) [6–8]. Reaction of lupane 3-oxo-2-ketenedithioacetal with hydrazine or guanidine produced substituted pyrazoles and pyrimidines [9]. A large series of indole derivatives of lupane-type triterpenoids was recently prepared by Fisher condensation of betulonic and dihydrobetulonic acids with arylhydrazines. High anticancer activity against various cell lines that was not known earlier was found for 2,3-indole-containing lupane-C-28-carboxylic acids. The most active of them were indoles with an unsubstituted N atom. The only compound containing a pyrrole ring annelated to a triterpene skeleton was the *N*-benzyl-4-phenylpyrrole derivative obtained from dihydrobetulonic acid condensed with benzylamine that underwent subsequent Michael reaction to form an imine with $PhCH{=}CHNO_2$ [10].

The pyrrole ring is a key element of fundamentally vital natural compounds such as chlorophyll, hemoglobin, vitamin B12, factor 430, biologically active compounds, and many drugs [11]. The development of effective synthetic methods for hybrid pentacyclic triterpenoids with a pyrrole ring opens the door to biologically active compounds with new and improved pharmacological properties.

An effective method for forming a pyrrole ring is Trofimov nucleophilic addition of ketoximes to acetylene in superbase media. This makes available a large number of previously unknown pyrroles and *N*-vinylpyrrole compounds of various structures [12–14], including steroids. The ability to use the method to synthesize pyrrole-containing steroids was studied using progesterone oximes, Δ^5-pregnen-3β-ol-20-one and Δ^5-cholesten-3-one as examples [15–17].

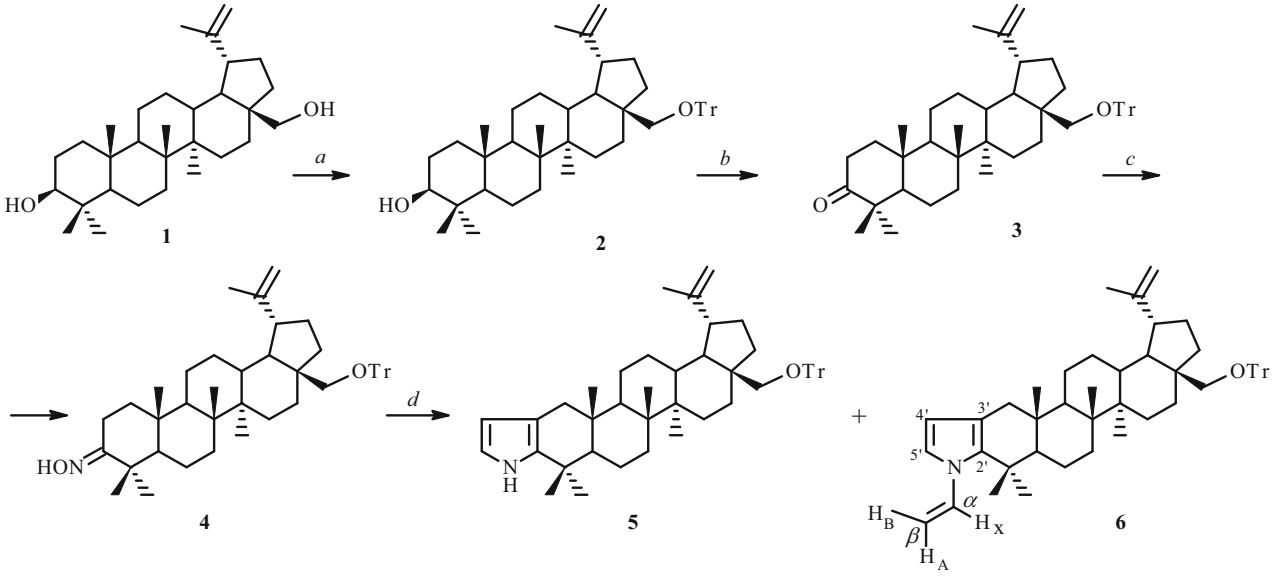
Herein we describe the first example of a Trofimov reaction of pentacyclic triterpenoids. Oximes of 3-keto derivatives of lupane-type pentacyclic triterpenoids are convenient subjects for studying the Trofimov reaction. They are available and are formed as a single isomer. The presence in one of the α-positions of a bulky *gem*-dimethyl substituent prevents the formation of regioisomers of the pyrrole rings. Betulin (**1**) was selectively protected at the primary hydroxyl by tritylchloride (TrCl). The resulting compound **2** was oxidized to ketone **3**, which was converted using $NH_2OH{\cdot}HCl$ to oxime **4**, which was then used as starting material. The trityl derivative **4** was selected because the trityl group is stable under superbase reaction conditions.

1) Ufa State Academy of Economics and Service, 450078, Ufa, Ul. Chernyshevskogo, 145, Russia; 2) Institute of Organic Chemistry, Ufa Scientific Center, Russian Academy of Sciences, 450054, Ufa, fax: (347) 235 60 66, e-mail: msyunusov@anrb.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 771–774, November–December, 2010. Original article submitted August 26, 2010.

 0009-3130/11/4606-0906

TABLE 1. Influence of Conditions for Reaction of Oxime **4** and Acetylene on Yields of **5** and **6** (P = 1 atm, KOH/DMSO)

4:DMSO:KOH ratio	T, °C	Time, min	Yield, %	
			5	**6**
1:330:10	65	15	41	15
1:330:5	80	50	46	13
1:100:5	80	25	60	14
1:100:5	80	240	–	13



a. CPh_3Cl, 4-DMAP, DMF, reflux [19]; *b*. 2 eq. PCC, CH_2Cl_2 [19]; *c*. $NH_2OH{\cdot}HCl$, Py, MeOH, reflux; *d*. acetylene, KOH/DMSO

Reaction of ketoxime **4** with acetylene in KOH/DMSO superbase medium occurred at atmospheric pressure to form primarily two compounds, pyrrole **5** and *N*-vinylpyrrole **6**. These were obtained pure by column chromatography over Al_2O_3. The influence of the reagent ratio and reaction conditions on the yields of **5** and **6** was studied (Table 1). Increasing the amount of base enabled the temperature to be lowered and the reaction time to be shortened practically without affecting the yields of **5** and **6** (Table 1). The degree of dilution turned out to be the most significant factor in this case. For a **4**:DMSO ratio of 1:100, the product yield after chromatographic purification was 60 (**5**) and 14% (**6**). Increasing the reaction time until complete consumption of pyrrole **5** (TLC monitoring) in order to increase the yield of *N*-vinylpyrrole derivative **6** led to polymerization of the reaction mixture and an increasing fraction of side products that could not be identified. The yield of **6** in this case was <13% (Table 1). Carrying out the reaction at elevated pressure in an autoclave (P = 16 atm, 1 h) also caused extensive polymerization that hindered isolation of **6**, which was formed in minor amounts according to the PMR spectrum.

The PMR spectrum of **5** contained the whole set of characteristic resonances for the pyrrole protons, i.e., two 1H doublets of doublets at 5.70 ppm (H-4′) and 6.51 (H-5′) and a broad singlet at 7.73 (NH). Resonances in the ^{13}C NMR spectrum of the pyrrole sp^2 C atoms were observed at 107.53 (C-4′), 113.71 (C-3′), 115.83 (C-5′), and 133.90 (C-2′). Three 1H resonances at 4.57 (d, $^3J_{AX}$ = 8.6, H_A), 4.98 (d, $^3J_{BX}$ = 15.5, H_B), and 7.17 (dd, $^3J_{BX}$ = 15.5; $^3J_{AX}$ = 8.6, H_X) in the PMR spectrum of **6** indicated that the structure contained an *N*-vinylpyrrole group. This moiety gave resonances at 97.84 (*N*-vinyl, C-β) and 133.41 (*N*-vinyl, C-α) in the ^{13}C NMR spectrum.

Thus, the ability to use the Trofimov reaction to synthesize pyrrole derivatives of lupane-type pentacyclic triterpenoids, which are difficultly accessible by other methods, was demonstrated for the first time using 28-triphenylmethyloxylup-20(29)-en-3-one oxime as an example. These compounds are potential biologically active compounds and promising intermediates for further modifications.

EXPERIMENTAL

PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at room temperature without a standard on a Bruker AM-300 spectrometer at operating frequencies 300 and 75 MHz, respectively. Chemical shifts are given in ppm relative to the solvent resonances δ_H 7.27 and δ_C 77.1 ppm. Resonances in ^{13}C NMR spectra were assigned using JMOD mode and heteronuclear correlation for direct SSCC (J = 140 Hz) (for **5**). Rotation angles were measured on a Perkin–Elmer 141 instrument. Mass spectra were recorded using direct sample introduction into the ion source on a Thermo Finnigan MAT 95XP spectrometer with temperature programmed from 50 to 270°C and ionizing potential 70 eV. Column chromatography was carried out over Al_2O_3 (Brockmann neutral) or SiO_2 (L grade, 100/160 mesh, Russia). TLC used Sorbfil plates (PTSKh-AF-A, Russia, Krasnodar, ZAO Sorbpolimer). Melting points were determined on a Kofler block. Betulin (**1**) was isolated from birch bark by the literature method [18]. Alcohol **2** and ketone **3** were prepared as before [19]. DMSO was stored for 12 h over KOH, stirred with CaH_2 for 2 d, and vacuum distilled over CaH_2 under an Ar atmosphere [20].

28-Triphenylmethyloxylup-20(29)-en-3-one Oxime (4). A solution of **3** (1.87 g, 2.74 mmol) was treated with $NH_2OH{\cdot}HCl$ (1.82 g, 26.28 mmol) in anhydrous MeOH (25 mL) and anhydrous Py (10.0 mL) and refluxed for 4 h. The solvents were evaporated. The solid was chromatographed over SiO_2 (benzene) to afford **4** (1.7 g, 89%), mp 148–151°C, [α] +1.9° (c 1.53, $CHCl_3$).

PMR spectrum (δ, ppm, J/Hz): 0.52 (3H, s, CH_3-27), 0.87 (3H, s, CH_3-25), 0.89 (3H, s, CH_3-26), 1.04 (3H, s, CH_3-24), 1.13 (3H, s, CH_3-23), 1.65 (3H, s, CH_3-30), 1.67 (1H, m, H-18), 2.22 (2H, m, H_A-2, H-19), 2.92 (1H, m, H_B-2), 2.93 (1H, d, J = 8.5, H_A-28), 3.15 (1H, d, J = 8.5, H_B-28), 4.53 (1H, s, H_A-29), 4.59 (1H, s, H_B-29), 7.20-7.45 (9H, m, H_{Ar}), 7.50 (6H, d, J = 8.0, H_{Ar}), 8.45 (1H, br.s, NOH).

^{13}C NMR spectrum (δ, ppm): 14.65 (q, C-27), 15.84 (q, C-25), 15.88 (q, C-26), 17.17 (t, C-2), 19.05 (t, C-6), 19.15 (q, C-30), 21.00 (t, C-11), 22.96 (q, C-23), 25.22 (t, C-12), 26.95 (t, C-15), 27.20 (q, C-23), 29.95 (t, C-21), 30.16 (t, C-16), 34.00 (t, C-7), 36.30 (t, C-22), 37.21 (s, C-10), 37.36 (d, C-13), 38.76 (t, C-1), 40.36 (s, C-4), 40.70 (s, C-8), 42.58 (s, C-14), 47.64 (s, C-17), 47.81 (d, C-19), 48.89 (d, C-18), 49.92 (d, C-9), 55.49 (d, C-5), 59.57 (t, C-28), 85.89 (s, O$\underline{C}Ph_3$), 109.47 (t, C-29), 126.34 (d, Ph C-4″), 127.30, 127.79, 128.01, 128.84 (all d, Ph C-2″, C-3″, C-5″, C-6″), 144.54 (s, Ph C-1″), 150.66 (s, C-20), 167.21 (s, C-3).

Reaction of 4 with Acetylene. A mixture of **4** (0.15–0.25 g, 0.22–0.36 mmol), KOH·0.5 H_2O (0.07–0.12 g, 1.08–1.79 mmol), and DMSO (50-25 mL) was stirred, purged with acetylene with heating (until complete consumption of starting oxime according to TLC), cooled, diluted with icewater (30 mL), and extracted with methyl-*tert*-butylether (5 × 30 mL). The combined extracts were washed with water and dried over Na_2SO_4. The solvent was evaporated. The solid was chromatographed over Al_2O_3 (eluent toluene:CCl_4, 2:1). Table 1 lists the temperature, time, and yields of **5** and **6**.

Pyrrolo[2,3-*b*]-28-triphenylmethyloxylup-20(29)-ene (5). Mp 182–183°C, [α] +3.5° (*c* 0.40, $CHCl_3$).

PMR spectrum (δ, ppm, J/Hz): 0.45 (1H, d, J = 14.0, H-5), 0.48 (3H, s, CH_3-27), 0.65 (3H, s, CH_3-26), 0.80 (3H, s, CH_3-25), 0.95 (3H, s, CH_3-24), 1.04 (3H, s, CH_3-23), 1.35 (2H, m, H_A-21, H_A-22), 1.54 (3H, s, CH_3-30), 1.57 (1H, m, H-18), 1.85 (1H, d, J = 14.9, H_A-1), 2.10 (3H, m, H-16, H-19, H_B-22), 2.38 (1H, d, J = 14.9, H_B-1), 2.82 (1H, d, J = 8.6, H_A-28), 3.03 (1H, d, J = 8.6, H_B-28), 4.42 (1H, s, H_A-29), 4.49 (1H, s, H_B-29), 5.70 (1H, dd, J = 2.5, pyrrole H-4′), 6.51 (1H, dd, J = 2.5, pyrrole H-5′), 7.20-7.70 (9H, m, Ph), 7.38 (6H, d, J = 7.6, Ph), 7.66 (1H, s, NH).

^{13}C NMR spectrum (δ, ppm): 14.32 (q, C-27), 15.64 (q, C-26), 16.14 (q, C-25), 19.09 (q, C-30), 19.18 (t, C-6), 21.16 (t, C-11), 23.66 (q, C-24), 25.28 (t, C-12), 27.00 (t, C-15), 29.88 (t, C-21), 30.09 (t, C-16), 31.10 (q, C-23), 33.57 (t, C-7), 33.62 (s, C-10), 35.22 (t, C-22), 37.40 (d, C-13), 38.46 (s, C-4), 39.36 (t, C-1), 40.60 (s, C-8), 42.46 (s, C-14), 47.58 (s, C-17), 47.71 (d, C-19), 48.83 (d, C-18), 48.98 (d, C-9), 53.12 (d, C-5), 59.52 (t, C-28), 85.80 (s, O$\underline{C}Ph_3$), 107.53 (d, pyrrole C-4′), 109.35 (t, C-29), 113.71 (s, pyrrole C-3′), 115.83 (d, pyrrole C-5′), 126.77 (d, Ph C-4″), 127.23, 127.69, 127.89, 128.76 (all d, Ph C-2″, C-3″, C-5″, C-6″), 133.90 (s, pyrrole C-2″), 144.47 (s, Ph C-1″), 150.79 (s, C-20).

Mass spectrum (*m*/*z*): found 705.487; calcd for $C_{51}H_{63}NO$ $[M]^+$ 705.490.

***N*-Vinylpyrrolo[2,3-*b*]-28-triphenylmethyloxylup-20(29)-ene (6).** Amorphous, [α] –2.0° (*c* 0.15, $CHCl_3$).

PMR spectrum (δ, ppm, J/Hz): 0.57 (3H, s, CH_3-27), 0.77 (3H, s, CH_3-26), 0.91 (3H, s, CH_3-25), 1.25 (3H, s, CH_3-24), 1.29 (3H, s, CH_3-23), 1.64 (3H, s, CH_3-30), 1.67 (1H, m, H-18), 1.95 (1H, d, J = 15.5, H_A-1), 2.15 (3H, m, H-16, H-19, H_A-22), 2.33 (1H, d, J = 15.5, H_B-1), 2.92 (1H, d, J = 8.2, H_A-28), 3.17 (1H, d, J = 8.2, H_B-28), 4.52 (1H, s, H_A-29), 4.57 (1H, d, $^3J_{AX}$ = 8.6, *N*-vinyl H_A), 4.59 (1H, s, H_B-29), 4.98 (1H, d, $^3J_{BX}$ = 15.5, *N*-vinyl H_B), 5.82 (1H, dd, J = 2.5, pyrrole H-4′),

6.83 (1H, dd, J = 2.5, pyrrole H-5′), 7.17 (1H, dd, $^3J_{BX}$ = 15.5, $^3J_{AX}$ = 8.6, *N*-vinyl H_X), 7.20–7.40 (9H, m, H_{Ar}), 7.52 (6H, d, J = 7.2, H_{Ar}).

^{13}C NMR spectrum (δ, ppm): 14.82 (q, C-27), 15.62 (q, C-26), 16.04 (q, C-25), 19.19 (q, C-30), 19.29 (t, C-6), 21.30 (t, C-11), 21.96 (q, C-24), 25.39 (t, C-12), 27.07 (t, C-15), 29.98 (t, C-21), 31.00 (q, C-23), 30.16 (t, C-16), 33.56 (t, C-7), 34.75 (s, C-10), 35.22 (t, C-22), 37.53 (d, C-13), 37.99 (s, C-4), 40.16 (t, C-1), 40.62 (s, C-8), 42.55 (s, C-14), 47.69 (s, C-17), 47.81 (d, C-19), 48.91 (d, C-18), 49.26 (d, C-9), 55.27 (d, C-5), 59.52 (t, C-28), 85.90 (s, O<u>C</u>Ph_3), 97.84 (t, *N*-vinyl C-*β*), 109.26 (d, pyrrole C-4′), 109.45 (t, C-29), 117.07 (s, pyrrole C-3′), 117.07 (d, pyrrole C-5′), 126.88 (d, Ph C-4″), 127.79 (d, Ph C-3″, C-5″), 128.86 (d, Ph C-2″, C-6″), 133.41 (d, *N*-vinyl C-*α*), 133.58 (s, pyrrole C-2′), 144.57 (s, Ph C-1″), 150.90 (s, C-20).

Mass spectrum (*m*/*z*): found 731.548; calcd for $C_{53}H_{65}NO$ $[M]^+$ 731.506.

ACKNOWLEDGMENT

We sincerely thank staff members of A. E. Favorskii Irkutsk Institute of Chemistry, Siberian Branch, Russian Academy of Sciences, Prof. A. I. Mikhaleva and Dr. A. M. Vasil′tsov for consultations in preparation for the experiments. The work was supported financially by the Presidium of the Russian Academy of Sciences (Basic Research Program No. 18P and No. 7) and the RF President (Grant for Leading Scientific Schools NSh-3756.2010.3).

REFERENCES

1. M. B. Sporn, K. Liby, M. M. Yore, N. Suh, A. Albini, T. Honda, C. Sundararajan, and G. W. Gribble, *Drug Dev. Res.*, **68**, 174 (2007).
2. J. Sejbal, J. Klinot, J. Protiva, and A. Vystrcil, *Collect. Czech. Chem. Commun.*, **51**, 118 (1986).
3. B. P. Pradhan and P. Ghosh, *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.*, **32**, 1068 (1993).
4. A. V. Korovin and A. V. Tkachev, *Izv. Akad. Nauk, Ser. Khim.*, 292 (2001).
5. M. Urban, J. Sarek, M. Kvasnica, I. Tislerova, and M. Hajduch, *J. Nat. Prod.*, **70**, 526 (2007).
6. Hya-Ok Kim, G. A. Tolstikov, and V. S. Bazalitskaya, *Zh. Obshch. Khim.*, **40**, 492 (1970).
7. Hya-Ok Kim, G. A. Tolstikov, and M. I. Goryaev, *Izv. Akad. Nauk Kaz. SSR, Ser. Khim.*, **19**, No. 6, 49 (1970).
8. L. R. Nigmatullina, O. B. Flekhter, L. A. Baltina, N. I. Medvedeva, and G. A. Tolstikov, *Khim. Prir. Soedin.*, 458 (2002).
9. K. Roy, K. Raj, and A. P. Bharduri, *Indian J. Chem., Sect. B*, **37**, 774 (1998).
10. V. Kumar, N. Rani, P. Aggarwal, V. K. Sanna, A. T. Singh, M. Jaggi, N. Joshi, P. K. Sharma, R. Irchhaiya, and A. C. Burman, *Bioorg. Med. Chem. Lett.*, **18**, 5058 (2008).
11. C. T. Walsh, S. Garneau-Tsodikova, and A. R. Howard-Jones, *Nat. Prod. Rep.*, **23**, 517 (2006).
12. B. A. Trofimov and A. I. Mikhaleva, *Khim. Geterotsikl. Soedin.*, 1299 (1980)
13. B. A. Trofimov, in: *Advances in Heterocyclic Chemistry*, A. R. Katritzky, ed., Academic Press, **51**, 177 (1990).
14. B. A. Trofimov and N. K. Gusarova, *Usp. Khim.*, **76**, 550 (2007).
15. A. M. Vasil′tsov, E. Yu. Shmidt, A. I. Mikhaleva, A. V. Afonin, and A. B. Zaitsev, *Khim. Geterotsikl. Soedin.*, 1641 (2001).
16. A. M. Vasil′tsov, A. B. Zaitsev, A. I. Mikhaleva, E. Yu. Shmidt, and A. V. Afonin, *Khim. Geterotsikl. Soedin.*, 66 (2002).
17. A. B. Zaitsev, A. M. Vasil′tsov, E. Yu. Shmidt, A. I. Mikhaleva, A. V. Afonin, and L. N. Il′icheva, *Zh. Org. Khim.*, **39**, 1479 (2003).
18. M. S. Yunusov, N. G. Komissarova, and N. G. Belenkova, RF Pat. No. 2,270,201 (2006); *Byull. Izobret.*, No. 5 (2006).
19. K. Hata, K. Hori, and S. Takahashi, *J. Nat. Prod.*, **65**, 645 (2002).
20. D. R. Burfield and R. H. Smithers, *J. Org. Chem.*, **43**, 3966 (1978).